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A MALARIA SURVEY OF LIBERIA

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Under the auspices of the U. S. Public Health Service and the Republic of Liberia, a survey to determine the incidence of malaria in Liberia was made between the months of May and October, 1948. Liberia lies on the west coast of Africa, approximately between 4°22′ and 8°50′ north latitude and between 7°30′ and 11°32′ west longitude. The estimated area is 43,000 square miles. It is bounded on the south by the Atlantic Ocean, on the west by Sierra Leone, on the north by French Guinea, and on the east by the French Ivory Coast.

The climate is equatorial. The annual rainfall ranges from about 180 inches on the coast to about 90 inches in the northern part. Most of the rain falls during the months of June, July, August, and September.

The coast is generally low and flat although it is broken by several capes upon which the largest cities of the country are located. Foothills appear a few miles inland, and these gradually increase in height as one proceeds northward. Mountainous areas occur in the central and northern parts of Liberia.

The soil is of lateritic origin which predisposes to quick absorption and rapid drainage of the rain water.

Most of the inhabitants are Negro aborigines who belong to a large number of tribes. Each tribe has its own ethnological distinctions and language or dialect. A smaller group of residents are descendants of Negroes returned from America, and these are known as "Americo-Liberians." The members of this small group are the best educated of the inhabitants and supply virtually all the leaders of the country.

The native houses are of stick and mud construction with thatch roofs and contain from one to several rooms. In the coastal cities, there are European-type houses usually built of wood and concrete with metal roofs.

METHODS

Upon entering a school or a village, the people were assembled and examined rapidly by two teams of survey personnel. A team consisted of: a clerk who recorded

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the age, sex, and name and assigned a number to correspond to a numbered slide; one member who cleansed the finger with a sponge soaked with 70 per cent alcohol; one man who punctured the finger and made the smear; and the last man who wiped the finger. By this method, the two teams could make an average of 225 smears per hour.

An effort was made to examine a large number of children by visiting schools. Often, however, the whole population of a native village would be tested, and parents were urged to bring the children, especially the infants. Blood from babies was usually taken from the big toe.

On several occasions, the blood smears were taken at night. It was often difficult to make smears after dark due to the swarms of insects which were attracted by the bright light of the kerosene lanterns. These insects frequently fell into the undried thick smears.

Thick and thin blood smears were made of each person examined; these slides were stained with Giemsa as soon as possible, always within a few days after having been made. Although some of the slides were examined in Liberia, the majority were returned to our U. S. Public Health Service laboratories in the United States for examination. The thick blood smears were examined routinely. In cases of doubt as to the specific identification of parasites, the thin smear was examined also. When *Plasmodium vivax* or *P. malariae* were found, the thin smear was also searched to determine if *P. ovale* were present. Parasites were identified as to species, except when not enough specific characters could be found.

At the time of microscopic examination, the density of the parasites was determined. Dr. Hildrus A. Poindexter (personal communication) has shown that the average white blood cell count of a large group of Liberians varied betwen 6100 and 7290 per cmm. We arbitrarily selected the volume of blood containing 650 w.b.c. as representing 0.1 cmm. of blood. The number of parasites in an area of the smear containing 650 w.b.c. was counted and multiplied by 10 to arrive at the number of parasites per cmm. Although it is realized that this method is not as accurate as might be desired, it seemed to be the best under the circumstances to approximate the relative parasite densities.

During the survey, over 10,000 blood smears were made. An effort was made to sample representative population areas of the country. The areas where surveys were conducted are shown in figure 1, and the types of population in the particular localities are listed below.

Coastal

Monrovia: Students at the following schools: Kindergarten, Government Morning, Trinity Parish, Bassa Community, Government Afternoon, Seventh Day Adventist, Edwin Barclay, J. B. McCritty, Haywood Industrial, Brook's Memorial, J. Z. Wilson Afternoon, C. B. Dunbar Memorial, J. J. Robert's Foundation Night, St. Teresa's Convent, St. Patrick's, Liberia College, and the College of West Africa.

Clara Town: Natives of the village.

Robertsfield: The natives of the villages of Peabody, Dodotown, Smell-No-Taste, Ketepu, Barkai, and Zaza.

Marshall: Natives of the village.

Harper: Children at the following schools: Harper Afternoon, Cape Palmas High, A.M.E. Day, St. Mark's Parish, St. Theresa's, Kru Community, and Bassa Community.

Robertsport: Children at the Episcopal Mission School, Government Morning and Afternoon Schools, and natives of the village of Grassfield.

FIG. 1. An outline map of Liberia showing the towns where surveys were made.

Central

Kakata: Children at the Government Baby Clinic and the following schools: Government Morning, Samuel D. Ferguson Memorial, Booker Washington Industrial, and the Catholic Mission.

Salala: Natives of the village.

Gbanga: Natives of the village.

Tappita: Children at the Mid-Liberia Mission School, Government Schools, and natives of the village.

Zoule: Natives of the village.

Northern

Ganta: Students at the Methodist Mission School and natives of the villages of Ganta (Gompa) and of the neighboring villages of Gboli, Gbuii, and Mganbe.

Flumpa: Children at the Flumpa Mission School and natives of the village.

Sanokwelle: Students at the Government School, Catholic Mission School, troops at the Soldiers' Barracks, and natives of the village.

Western

Vonjama: Children at the Government School and natives of the village and the neighboring village of Malamai.

Salaga: Natives of the village.

Zorzor: Children at the Luthern Mission School and natives of the village.

Eastern

Tchien: Children at the Assembly of God School, Government Schools, and natives of the village.

OBSERVATIONS

Parasite Incidence

There were 10,128 blood smears examined, of which 3,104 (30.65 per cent) showed malaria parasites. The data relative to the malaria incidence according to area are shown in table 1. For purposes of comparison, the country was divided into five areas (see table 1). The coastal area had less malaria than the interior. The differences between the coastal region and the other areas were highly significant. The critical ratios between these areas and the coast are as follows: Central, 10.42; Northern, 19.29; Western, 11.72; Eastern, 7.80; and the combined interior areas, 20.62.

Sex. Nearly twice as many males as females were examined (table 2, figure 2). The males showed a higher rate of malaria (31.62 per cent) than the females (28.89 per cent), and this difference was significant (standard errors 2.89). The male sex had a significantly higher malaria rate in the age groups 5–9, 10–14, and 15–19. In the other age groups, there was no significant difference between the sexes.

Age. The highest incidence of malaria was found in the age group 1–4 years (tables 2, 3, 4, figure 2). In the youngest age group, that under one year, 43.88 per cent of those tested were infected. This incidence increased to the peak of 64.45 per cent in the group 1–4 years, and after that it dropped steadily to the 15–19 years age group. The differences between any age group and the group immediately preceding or following it was significant except for the 15–19 years and the over 19 years groups.

In table 1, it is shown that in general the interior areas had more malaria than the coastal region. Table 4 shows that this is true in each age group. Tests of significance were run on the total ages, on the age group (1–4) having the most malaria, and on the age group (over 19) having the least malaria. In each instance, the malaria rate was significantly lower on the coast than in any interior area for any particular age group.

Species of malaria. Plasmodium falciparum was the predominant parasite not only for the population as a whole (*falciparum* comprised 83.8 per cent of all diagnosed) but also in each area and in each age group (tables 3, 5).

P. malariae made up 14.2 per cent of the infections found. It was most prevalent in the children under five years of age, amounting to approximately 20 per cent of the positive cases.

P. vivax accounted for only 2.0 per cent of the total infections, and it was more frequently found in those under five years of age.

P. ovale appeared to be present in only one case, an adult male.

In about one-tenth of the positive cases, specific indentification of the parasites could not be made.

TABLE 1

Incidence of malaria in Liberia by areas and towns

AREA AND TOWN	MONTH OF SURVEY	TOTAL		
		Examined	Positive	Per cent Positive
Coast				
Monrovia	June	2,579	481	18.65
Clara Town	September	201	63	31.34
Robertsfield	July	616	96	15.58
Marshall	July	468	134	28.63
Harper	September	500	145	29.00
Robertsport	September	554	122	22.02
Total		4,918	1,041	21.17
Central				
Kakata	July	313	138	44.09
Salala	July	455	196	43.08
Gbanga	July	497	236	47.48
Tappita	August	521	81	15.55
Zoule	August	218	26	11.93
Total		2,004	677	33.78
Northern				
Ganta	July	595	271	45.55
Flumpa	July	171	73	42.69
Sanokwelle	July	689	367	53.27
Total		1,455	711	48.87
Western				
Vonjama	June	483	201	41.61
Salaga	June	159	53	33.33
Zorzor	July	434	176	40.55
Total		1,076	430	39.96
Eastern				
Tchien	August	675	245	36.30
Grand Total		10,128	3,104	30.65

In the population as a whole, the incidence of the different species of malaria was: 23.90 per cent of the people had *P. falciparum;* 4.06 per cent had *P. malariae;* 0.57 per cent had *P. vivax;* 0.01 per cent had *P. ovale;* and 3.19 per cent had infections which were not diagnosed as to species.

Mixed infections. Of the malaria cases diagnosed as to species, 3.8 per cent had multiple infections (table 3). There were 100 cases with *P. falciparum* and *P. malariae*, 9 with *P. falciparum* and *P. vivax*, 1 with *P. falciparum* and *P. ovale*, and 1 with *P. malariae* and *P. vivax*. The multiple infections occurred more frequently in those under five years of age and tended to be found less frequently with increase in age.

The proportion of the positive cases showing multiple infections in each age group was: under 1 year, 11.63 per cent; 1–4 years, 9.44 per cent; 5–9 years, 3.24 per cent; 10–14 years, 2.53 per cent; 15–19 years, 3.41 per cent; and over 19 years, 1.92 per cent. Of the total 3,104 positive cases, including the undetermined species, 3.58 per cent had multiple infections.

Intensity of infections. The parasite densities ranged from 1 to over 100,000 per cmm. One child less than one year of age, infected with *P. falciparum*, showed more

TABLE 2

Incidence of malaria by age groups and sex showing the differences between the sexes

AGE GROUP	MALE			FEMALE			BOTH SEXES		CRITICAL RATIOS BETWEEN SEXES
	Examined	Per cent Positive	Standard Deviation	Examined	Per cent Positive	Standard Deviation	Examined	Per cent Positive	
years									
Under 1	43	51.16	7.62	55	38.18	6.55	98	43.88	1.29
1–4	265	62.64	2.97	261	66.28	2.93	526	64.65	0.87‡
5–9	1,021	52.32	1.56	598	44.82	2.03	1,619	49.54	2.92†
10–14	1,335	37.23	1.32	605	29.09	1.85	1,940	34.69	3.59†
15–19	1,347	25.17	1.18	476	14.92	1.63	1,823	22.49	5.07†
Over 19	2,463	19.85	0.80	1,657	20.88	1.00	4,120	20.77	0.80‡
Unknown	—			—			2	100.00	
All Ages	6,474	31.62	0.58	3,652	28.89	0.75	10,128*	30.65	2.89†

* Includes two sex not given.

† In these age groups, the males had significantly more malaria than the females, using a critical ratio of 2 or above as indicating significance.

‡ These were the only age groups in which the females showed a higher incidence of malaria than the males and the difference was not significant.

than 100,000 parasites per cmm. Most of the positive cases had parasite densities of less than 100 per cmm. Due to the asymmetrical distribution of the parasite densities, table 6 has been prepared to show the medians and the arithmetic and geometric means, all by age groups. The arithmetic means have been plotted in figure 3. Graphs of the geometric means show a similar type of curve except that the mean values are smaller.

P. malariae was similar to *P. falciparum* in that it showed the greatest density of parasites in the youngest age group and the lowest density in the 10–14 years age group. The average density for all of the age groups was lower than the average *P. falciparum* density.

P. vivax likewise showed the highest density in the age group under 1 year and the lowest density in the 10–14 years age group. Although in the group under one year

of age, *P. vivax* had more parasites per cmm. than *P. malariae*, in every other age group *P. malariae* showed a greater density. The average density for all *P. vivax* infections was very similar to that of *P. malariae*.

As a rule, *P. malariae* causes more ill effects clinically than *P. vivax* for the same parasite density. This fact plus the higher incidence of *P. malariae* in the population

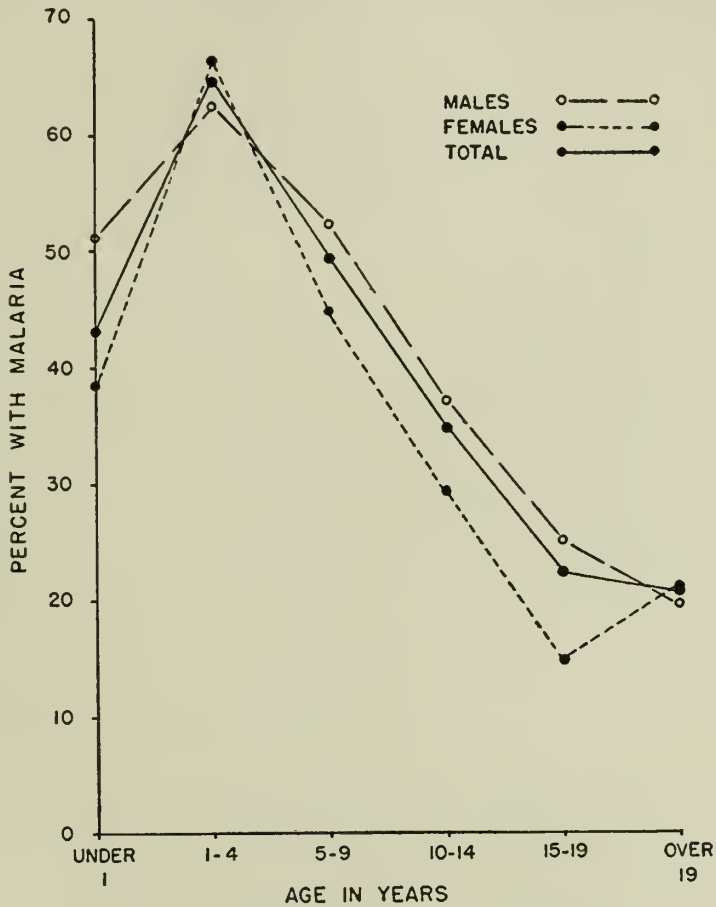

FIG. 2. The incidence of malaria by age groups and sex

(estimated seven times as prevalent) makes *P. malariae* of greater importance in Liberia than *P. vivax*.

Gametocytes of P. falciparum. Of the 2,421 *P. falciparum* infections, 12.06 per cent had gametocytes (table 7, figure 4). Gametocytes were present most commonly (26.47 per cent) in the youngest age group, then dropped gradually to 9.55 per cent in the 10–14 years age group, after which there was a slight increase in the percentage of infections with gametocytes.

A total of 2.88 per cent of the people examined had *P. falciparum* gametocytes in blood smears. As in the total parasite counts, *P. falciparum* gametocytes tended to

TABLE 3

Incidence of malaria in Liberia by age group and species of Plasmodium

PERSONS			PLASMODIUM SPECIES								TOTAL OF INDIVIDUAL SPECIES OF PLASMODIUM					
			Single Infections				Multiple Infections*				*falciparum*		*malariae*		*vivax*	
Age Groups	Examined	Per cent Positive	*falciparum*	*malariae*	*vivax*	Undetermined	FM	FV	FO	MV	Number	Per cent Persons Infected	Number	Per cent Persons Infected	Number	Per cent Persons Infected
years																
Under 1	98	43.88	29	5	1	3	4	1			34	34.69	9	9.18	2	2.04
1–4	526	64.45	240	39	7	21	29	3			272	51.71	68	12.93	10	1.90
5–9	1,619	49.54	627	84	10	55	23	3			653	40.33	107	6.61	13	0.80
10–14	1,940	34.69	538	61	9	48	16	1			555	28.61	77	3.97	10	0.52
15–19	1,823	22.49	304	43	12	37	13			1	317	17.39	57	3.13	13	0.71
Over 19	4,120	20.27	572	78	9	160	14	1	1		588	14.27	92	2.23	10	0.24
Unknown	2	100.00	1				1				2		1			
Total—All Ages	10,128	30.65	2,311	310	48	324	100	9	1	1	2,421	23.90	411	4.06	58	0.57

* FM—*P. falciparum* and *P. malariae.*
FV—*P. falciparum* and *P. vivax.*
FO—*P. falciparum* and *P. ovale.*
MV—*P. malariae* and *P. vivax.*

TABLE 4

The incidence of malaria in the different areas by age groups

AGE GROUP	PER CENT WITH MALARIA				
	Coast	Central	Northern	Western	Eastern
years					
Under 1	29.41	65.22	42.86	38.10	66.67
1–4	47.21	75.61	83.52	67.57	89.13
5–9	34.52	67.10	71.37	67.33	65.17
10–14	22.48	45.54	64.25	65.35	57.33
15–19	15.04	32.92	50.68	38.04	26.32
Over 19	11.41	19.27	32.66	25.00	21.14
All ages	21.17	33.78	48.83	39.96	36.30

TABLE 5

Ratio of plasmodia species according to age groups. The undetermined species are not included

AGE GROUPS	TOTAL INFECTIONS*	RATIO PER CENT		
		P. falciparum	*P. malariae*	*P. vivax*
years				
Under 1	45	75.6	20.0	4.4
1–4	350	77.7	19.4	2.9
5–9	773	84.5	13.8	1.7
10–14	642	86.4	12.0	1.6
15–19	387	81.9	14.7	3.4
Over 19	690	85.2	13.3	1.5
Ages unknown	3	66.7	33.3	0.0
All Ages	2,890	83.8	14.2	2.0

* The single *P. ovale* infection is not included.

TABLE 6

The average parasite density per cmm. of the malaria species showing the median and the arithmetic and geometric means, arranged by age groups

AGE GROUP	*P. falciparum*			*P. malariae*			*P. vivax*		
	Means		Median	Means		Median	Means		Median
	Arithmetic	Geometric		Arithmetic	Geometric		Arithmetic	Geometric	
years									
Under 1	9,841.8	1,060.06	825	465.6	264.47	290	1,810.0	378.42	A
1–4	2,070.7	387.97	360	338.5	111.65	105	156.0	104.11	180
5–9	489.6	159.57	140	159.4	77.52	80	125.4	69.46	50
10–14	278.6	91.09	75	71.4	38.27	30	42.0	31.13	30
15–19	335.9	92.03	80	109.5	39.10	30	101.7	25.12	B
Over 19	294.7	77.65	70	115.3	41.37	30	62.0	34.02	30
All Ages	698.1	125.26	100	163.5	58.71	50	159.1	48.97	40

A—Only 2 cases, one having 40 and one having 3,580 parasites per cmm.

B—There were 12 cases, 8 of which had 10 parasites per cmm. each.

concentrate in the lower density groups, resulting in an asymmetrical distribution. Of the 292 gametocyte counts, 176 showed fewer than 11 per cmm.; 107 had between

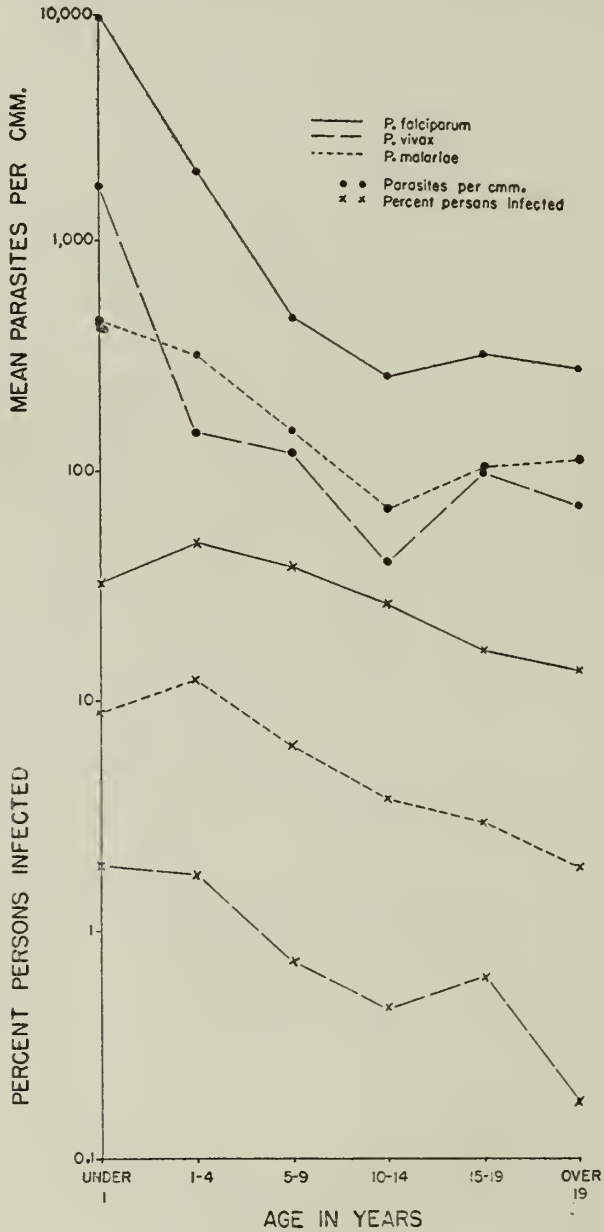

FIG. 3. The percentage of persons infected with each species of plasmodium, and the arithmetic means of the parasite densities of each species of plasmodium, by age groups.

11 and 100 per cmm.; 8 between 101 and 1,000 per cmm., and only one had over 1,000 per cmm. The younger age groups showed the highest density of gametocytes.

TABLE 7

The incidence of P. falciparum *gametocytes in the population, in* P. falciparum *infections, and the average gametocyte density*

AGE GROUP	PERSONS			P. FALCIPARUM INFECTIONS		
	Number Examined	Number with Gametocytes	Per cent with Gametocytes	Number	Per cent with Gametocytes	Gametocytes per cmm. Mean (Arithmetic)
years						
Under 1	98	9	9.18	34	26.47	40.0
1–4	526	48	9.13	272	17.65	33.1
5–9	1,619	69	4.26	653	10.57	71.7*
10–14	1,940	53	2.73	555	9.55	14.9
15–19	1,823	33	1.81	317	10.41	17.0
Over 19	4,120	80	1.94	588	13.61	22.9
All Ages	10,128†	292	2.88	2,421†	12.06	34.5

* One infection showed 3,320 per cmm. so that the remaining 68 infections averaged 22.7 pe cmm.

† Includes two whose age was not given.

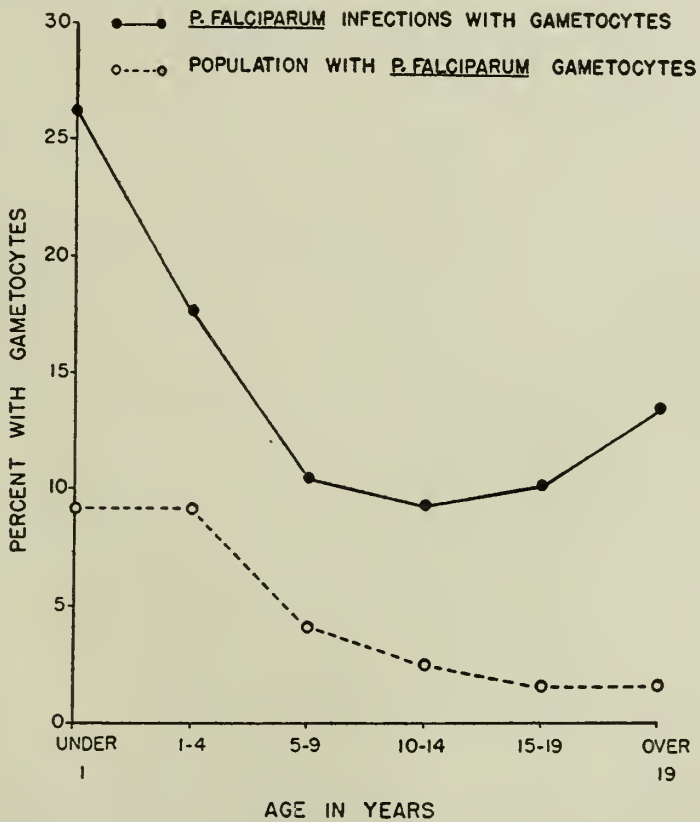

FIG. 4. The percentage of the population and of the *P. falciparum* infections showing *P. falciparum* gametocytes.

Mosquito Survey

Adult collections. Adult mosquitoes were sought in native houses, in crevices of large trees in the villages, in outhouses, such as toilets or sheds, and under bridges and culverts.

For examining the native houses, a flashlight was necessary because of the poor light. The wall areas, inside of the ceiling or roof, native mud beds, the sides of wooden beds, bed nets if present, and any other furniture were examind. When mosquitoes were found, they were sucked into an aspirator for later identification and examination for infection. If a house was fairly free from smoke, the mosquitoes were usually found at the top of the inside walls and on the ceilings. However, many houses had small fires in the center for cooking and for warmth; in some of these, the smoke became thick, particularly at the ceiling, and the mosquitoes moved down the wall, roughly in proportion to the amount of smoke present. In these houses, mosquitoes might be found also on the outside walls, just under the eaves of the thatched roof, or on the bottom side of the overhanging roof itself.

Toward the latter part of the survey, mosquitoes were collected by spreading sheets on the floor and over the furniture and releasing spray from a pyrethrum bomb. After doors and windows had been closed for five minutes, the mosquitoes which had been knocked down were collected from the sheets. This method was faster than aspiration, and more mosquitoes were collected, probably due to the fact that mosquitoes dropped from the thatched roofs where they could not be found by the aspiration method.

The main disadvantage of the spray method was that the mosquitoes were killed and, consequently, were not as good for dissection later as those that were caught alive.

The persons collecting mosquitoes were experienced and seldom overlooked any mosquitoes on the walls. Often the residents of the houses would become quite interested in searching for the mosquitoes and enter the search with enthusiasm, expressing evident pleasure when finding one.

The results of the adult collections are given in table 8. Most of the anopheline mosquitoes were taken from the houses, but most of the culicines were collected from other places, particularly toilets. During the estimated 100 man-hours spent on collections, 1,153 anopheline mosquitoes were taken. The number of anopheline mosquitoes in a house ranged from none to 50 or more and averaged 2.76 per house.

Of the 1,153 adult anopheline mosquitoes taken, 94.28 per cent were *A. gambiae;* 5.20 per cent were *A. funestus;* and 0.26 per cent each were *A. hancocki* and *A. nili.* Only 22 *A. gambiae* were found in places other than dwellings.

Of the 543 *A. gambiae* dissected to determine infections, 6.45 per cent showed oocysts, sporozoites, or both (table 9). Since the mosquitoes usually were engorged with blood, a fact which made dissection of the gut and detection of oocysts difficult, only salivary glands were dissected in the majority of cases.

Larvae collections. The places in which *A. gambiae* were found breeding are shown in table 10. As expected, small exposed areas of water near human habitations were often prolific breeding places of *A. gambiae.* Depressions made by man, especially if

TABLE 8

Adult mosquitoes collected

DATE 1948	LOCATION	HOUSES	TIME*	ANOPHELES				CULICINES
				gambiae	*funestus*	*hancocki*	*nili*	
			Hr: Min.					
6/29	Vonjama	5	2:15	12				
9/21	Robertsport							
	(Aspiration)	4	2:30	7				
	(Sbeet Collection)	4	0:40	10		1		
7/12	Sanokwelle	11	4:20	8	9			
7/12	Under bridge, Sanokwelle	Under bridge	1:15	9	10	1	1	2
7/12	Sanokwelle road	5 rock crevices	0:35	0				2
6/28	Ganta town	15	4:15	31				
6/29	Ganta town	15	4:20	57				
6/30	Ganta town	10	3:00	8				
6/30	Ganta town	5 toilets	1:40	5				157
7/7	Ganta town	10	3:45	35	1			
7/8	Ganta town	5	1:45	17				
7/17	Ganta town	20	5:15	9	8			
8/18	Tappita	7 toilets	3:30	8		1		Hundreds
6/29	L.C.C. Labor Camp, Flumpa	4	1:40	37				
7/9	L.C.C. Labor Camp, Flumpa	10	2:40	11				
7/13	L.C.C. Labor Camp, Flumpa	10	5:50	14				
7/13	Flumpa	6	2:10	3				
6/28	Wborle town, Ganta	3	1:15	3				
7/1	Ganta Mission	6 toilets	2:04	0				44
7/2	Ganta Mission	2	1:54		1			4
7/5	Whorle town, Ganta	8	2:05	3	1			
7/7	Leper Colony, Ganta	5	1:10		1			
7/10	Ganta Mission	10	3:00	14	4			
7/16	Gbesselah, Ganta	15	4:35	7				
7/19	Gbanga Headquarters	18	5:04	76	17		2	
7/20	Salala Headquarters	16	5:15	19	8			
9/1	Kakata	8	2:40	42				
9/2	Kakata	13	6:35	107				
9/3	Kakata	9	4:30	59				
7/22	Robertsfield	15	5:40	12				
7/22	Robertsfield, Dodo Town, Div. #45, Firestone	6	1:17	3				
7/23	Marsball	12	4:00	10				
8/17	Paynesville	6	3:10	109				
8/19	Paynesville	4	2:15	44				
8/25	Paynesville	5	2:15	30				
8/27	Paynesville	5	1:45	25				
7/28	Mount Barclay, Monrovia	20	7:15	144				
8/16	Zone #2, Monrovia	4	3:16	0				8
8/23	Congo town, Monrovia	10	4:30	5				
8/26	Zone #2, Monrovia	4	1:25	0				14
8/28	Busbrod Island, Monrovia	18	5:10	7				22
8/30	Sinkor, Monrovia	12	3:15	19				
8/31	Congo town, Monrovia	16	2:25	42				
9/10	Harper†	12	5:00	23				
8/22	Tchien	18	6:30	3				
Total—Houses		403		1,065	50	1	2	
Total—All		428	99:50	1,087	60	3	3	

* Man-bours spent in collecting.

† Some by sbeet collection after spraying

recently associated with human contacts, appeared to be favorite breeding places; these included excavations for mud for house construction, footprints, wheel ruts, and particularly the tracks left by roadbuilding machinery (figure 5c). A major problem in Monrovia is the native wells made by sinking oil drums into the ground. Often these places were heavy breeders of *A. gambiae* even when heavy fecal pollution was evident.

In some roadside ditches where water had dried up between rains leaving cracked mud, many larvae of *A. gambiae* appeared within a few minutes after water was added to the mud (figure 5d). It became apparent that the larvae were able to survive for sometime between rains in the crevices of the mud.

On Bushrod Island in the salt flats of Stockton Creek, only a few hundred feet from the road connecting the city of Monrovia to the port, larvae resembling *A. gambiae* were collected from brackish pools on several occasions (figure 5a, 5b). It occurred to us that these might be *A. melas*, and the following observations were made.

TABLE 9

A. gambiae *showing malaria infections*

MONTHS COLLECTED	LOCATION	NUMBER DISSECTED	NUMBER INFECTED
July	Ganta	5	1
September	Kakata	23	3
Aug.–Sept.–Oct.	Monrovia	503	31
August	Monrovia*	12	0
		543	35

* Collected from tree bases; all others from houses.

The chloride contents of these pools varied from about 3,500 ppm. during the middle of the rainy season to 12,000 ppm. at the end of the rainy season. This exceeds the salinity in which *A. gambiae* can survive (Ribbands, 1944). Some of the larvae were put into sea water, and although these survived, *A. gambiae* from fresh water did not.

Just a few yards from this breeding area is the village of Clara Town. *A. gambiae* was known to be breeding on the side of the village opposite the brackish area. Adult mosquitoes were collected from the tree bases in the village and induced to oviposit. Batches of eggs were put into brackish water from the breeding places. Eggs from some of the batches hatched, and the larvae developed normally to the adult stage. These we believed to be *A. melas*. Other batches of eggs hatched, but the larvae died immediately. These we believed to be *A. gambiae* from the fresh water side of the town.

The survival of larvae in sea water is held to be a good test of the species of *A. melas*. Ribbands (1944) claimed that *A. gambiae* larvae cannot live in a concentration of sea water 37.5 per cent or above. The British workers in Freetown, Sierra Leone, use survival in sea water as a quick way to differentiate between *A. gambiae* and *A. melas* (personal communication). Therefore, we believe that the mosquitoes we

TABLE 10

Breeding places of Anopheles gambiae*

DATE	LOCATION	NATIVE WELLS	ROADSIDE DITCHES	WHEEL RUTS	SMALL POOLS	EDGE OF LAKE	SWAMPS WITH WEEDS	SLOW DRAIN-AGES	SLOW STREAMS	POOLS EDGE OF SWAMP	SEEPAGE POOLS FROM HILL	MUD HOLES EXCA-VATED FOR HUTS
6/30/48	Vonjama									++		++++
7/12/48	Sanokwelle		++				++		++	++++		
7/17/48	Ganta				++		++	++				
7/19/48	Gbanga		++				++	++++	++			
7/20/48	Salala							++++	++			
7/23/48	Robertsfield		+++	++	++			++		+		
7/29/48	Monrovia	++++	++++	++++	++++	++		++	++			++
8/18/48	Tappita						++++					++++
8/22/48	Tchien		+++		+++							++++
9/2/48	Kakata		+++	++++	+++		++	++	++	++++		++++
9/10/48	Harper		+++							++++		
9/13/48	Robertsport				++		+++	+++			+++	

* The arbitrary scale of pluses ranges from +, indicating one or a few larvae, to ++++, indicating very many larvae. A blank space under a location indicates either that no larvae were found or that such a location was not examined in that village.

found breeding in the brackish water were *A. melas*. This appears to be the first report of *A. melas* in Liberia. Liberia is not listed as a locality for *melas* by deMeillon (1947). However, *A. melas* is found in the neighboring country of Sierra Leone where it has been studied carfully by the British workers.

We were able to determine neither the extent of *A. melas* along the coast nor the

FIG. 5

Upper Left.—The brackish water area on the edge of Stockton Creek where *A. melas* was found breeding.

Upper Right.—A close-up of a small pool in the edge of the area shown in Upper Left, in which larvae of *A. melas* were found. The next day this pool appeared dry, but upon addition of water, many larvae reappeared.

Lower Left.—Pools of rainwater in wheel ruts left by roadbuilding machinery. Some of these pools were prolific breeders of *A. gambiae*.

Lower Right.—Cracked mud in the roadside ditch of a newly constructed road. Upon addition of water to this mud, many larvae of *A. gambiae* appeared.

part it plays in the transmission of malaria. Muirhead-Thomson (1948) has shown that 3.5 per cent of *A. melas* dissected had sporozoites compared to 10.0 per cent of *A. gambiae*. If *melas* is found to be widely distributed along the coast of Liberia, and there is little reason to suspect otherwise, it will form a good, if less efficient partner for *A. gambiae* in the transmission of malaria.

A. smithii was found on Cape Mount, several hundred feet above the town of

Robertsport, in a small reservoir used to supply water to a nearby mission. The reservoir was fed by a cool mountain stream which was heavily shaded and which contained a considerable amount of dead vegetation. The larvae were numerous especially along the sides of the reservoir, and they could be found also at the entrance of the stream into the reservoir where the water was turbulent.

Nothing of particular interest was noticed in the breeding areas where other species of anophelines were collected.

DISCUSSION

As a result of a single blood smear examination, 31 per cent of the persons examined were found infected with malaria. Since it is known that single blood smear examinations do not reveal all of the infections, the actual rate probably is higher than the one shown. Apparently, malaria was acquired early in life. The survey showed that most of the children under five years of age exhibited parasites. The parasite density was highest, and the highest proportion of multiple infections occurred in this same group. After the age of five years, the incidence and the parasite density decreased gradually. The incidence found and the distribution of the parasite density by age indicate that persons are being infected continually from infancy. Those who survive the first four years develop an immunity that is effective enough to lower the patent parasitemia but not sufficient to prevent re-infection. It seems likely that every person is infected not only once but many times during his life.

Malaria is hyperendemic for the country as a whole although it is more prevalent in the interior. No single factor was apparent to explain the marked difference in the amount of malaria between the coastal and the interior regions, but it is probably due to a combination of housing, diet, breeding areas for mosquitoes and prevailing winds.

P. falciparum was the predominant species of malaria for all ages. Not only were its incidence and parasite density the highest in those under five years of age, but also the greatest proportion of gametocyte carriers occurred in this age group. These young children constitute the seed bed of malaria and undoubtedly are responsible for the majority of the transmission of malaria by mosquitoes.

P. malariae constitutes the second most important species of malaria in Liberia. Its competition for human hosts started early and accounted for two out of every ten of the malaria infections present in children under five years of age. As the hosts became older, the relative proportion of *P. malariae* to other species dropped to about 1.3 to 10; this loss was reflected largely by a gain in proportion of *P. falciparum*.

Of the 77 children under five years with *P. malariae*, 43 per cent also had *P. falciparum*. These two species were present together less frequently in persons above that age. Only 24 per cent of the total *malariae* infections were associated with *falciparum*. The relatively large proportion of *P. malariae* infections occurring concomitantly with *P. falciparum* in the youngest age groups suggests a lack of antagonism between these two species. The decreasing incidence of quartan malaria in the older age groups appears to be a result of host-parasite relationship rather than antagonism between these two species of parasites.

It has been shown that *P. vivax* is of lesser importance in Liberia than either *P. falciparum* or *P. malariae*.

P. ovale was rare; one adult male was found infected. All of the asexual forms found in the blood smear, including segmenters with eight merozoites within enlarged, stippled erythrocytes, were characteristic of *P. ovale*.

Previously, we have pointed out the possibility of *P. vivax* producing forms resembling *P. ovale* (Young and Eyles, 1949), particularly at the peak of the infection. However, in this instance, the infection was of a low density, and every parasite seen resembled *P. ovale*. Therefore, we believe this infection was *P. ovale* and not an aberrant form of *P. vivax*.

Previous reports of *P. ovale* in Liberia are rare. Barber, Rice, and Brown (1932) did not mention its presence in 635 malaria cases they examined although Doctor Barber believed he had earlier seen about a dozen *ovale* infections in Nigeria (Barber Olinger, and Putnam, 1931). Field (1941) did not mention Liberia in listing areas in which *P. ovale* had been reported.

The only report of *ovale* in Liberia that we can find is that of Moore (1945), who, in examining American troops stationed there, found one *ovale* case in 1,236 with malaria. No comment was made upon finding this relatively rare species except that one case was diagnosed. As far as can be determined from this report, only the thick blood films were examined. It is generally accepted that an accurate diagnosis of *P. ovale* from a thick film is virtually impossible.

It seems, from the evidence presented above, that *A. gambiae* is by far the most important vector of malaria in Liberia. In certain areas where the salt marsh breeding areas of *A. melas* adjoin the fresh water breeding areas of *A. gambiae*, the former may be shown to be an important ally and an additive factor in the transmission of malaria.

In the regions we visited, *A. funestus* comprised only 5.2 per cent of the total adult anophelines caught as compared to 94.3 per cent for *A. gambiae*. *A. funestus* has been found to be a good vector of malaria in Liberia (Barber *et al.*, 1932), but its relative scarcity, as compared to *A. gambiae*, would limit its importance as a vector during the wet season.

Barber *et al.* (1932) found a higher proportion of *A. funestus* (51.1 per cent of the total species) than we did. Their survey was done during the months of February through May. During May, which is the beginning of the rainy season, the relative proportion of *A. gambiae* had risen to 71.5 per cent of the total species as against 45.5 per cent during the entire four-month period. Evidently, the proportions of the malaria vectors present appear to depend upon the season. *A. gambiae* is most prevalent during the wet season, a fact which can be correlated directly with the greater abundance of small pools of water in which it prefers to breed.

Since we did not make observations throughout the entire year, it cannot be said definitely in which season most transmission of malaria occurs. Due to the abundance of vectors, it seems likely that transmission occurs throughout the year. It would appear that the greatest transmission occurs during the wet season when *A. gambiae* is most commonly present.

SUMMARY AND CONCLUSIONS

1. Blood smears were made in representative population centers of Liberia. Of the 10,128 blood smears examined, 30.65 per cent had malaria parasites.

2. Malaria was most prevalent in the 1–4 years age group, 64.45 per cent of which was positive. The greatest density of parasites was in the group under one year of age.

3. *Plasmodium falciparum* was the predominant species and accounted for 83.8 per cent of the diagnosed infections. A total of 14.2 per cent were *P. malariae*, and 2.0 per cent were *P. vivax*. *P. ovale* was found in one case.

4. Of the 2,421 *P. falciparum* infections, 12.06 per cent had gametocytes. The younger age groups showed the highest proportion of infections with gametocytes.

5. *Anopheles gambiae* was the most prevalent vector. Of 543 *A. gambiae* dissected, 6.45 per cent were infected with malaria.

6. *A. melas* was found breeding on the coast.

7. Malaria was hyperendemic throughout the country. The costal region was less heavily infected than the interior.

8. It seems apparent that the native population is bitten constantly by infected mosquitoes. Because of the larger number showing gametocytes and the greater intensity of the infections, persons under five years of age appear to constitute the seed bed of transmission.

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EFFECTIVENESS AGAINST FLIES AND MOSQUITOES OF DDT APPLICATIONS TO CLAY, PALM, AND STRAW SURFACES[1]

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Malaria control in rural areas is a great problem in India, and is particularly complex because of the low standards of living and the poor housing of the rural population. Prior to the discovery of DDT, periodic spraying with pyrethrum extract was found to be the only effective and comparatively inexpensive method of controlling malaria in South India (Russell and Knipe 1939–41). Because of the unique residual toxicity manifested by DDT when sprayed on the walls and ceilings of dwellings, much work has been done by many workers to determine the toxicity of DDT residue on various types of surfaces. This insecticide is now extensively used in India against anopheline mosquitoes for malaria control.

To determine the relative effectiveness of DDT when applied as a solution, emulsion, or suspension on building surfaces encountered in malarious rural areas of India, a series of tests were made on straw, palm-leaf, and clay-cow-dung panels. These surfaces broadly represented the types of materials that are being used for the roofs and walls of the huts in rural India. These experiments were conducted at the Orlando, Fla., laboratory of the Bureau of Entomology and Plant Quarantine.

Preparation of Panels.—The surfaces to be tested were all attached to 6- by 7-inch plywood panels. For the clay-cow-dung panels, a strip $\frac{1}{2}$ inch high was fastened around the edge of the plywood panel, thus forming a tray to retain the clay. Well-dried palm leaves were cut from stretched leaves and stapled to the panels to simulate thatched walls, and dried wheat straw similar to that used for thatching in India was fastened in bundles approximately $\frac{1}{2}$ inch thick and one inch wide to cover the surface of other panels. The straw was firmly secured to the panels by passing loops of twine around each bundle and tying the ends of the twine on the reverse side of the panels.

Clay-cow-dung panels were prepared with clay soil that had been well worked to remove stones, lumps, leaves, roots, and other extraneous materials. This wet clay was placed in trays $\frac{1}{2}$ inch deep and the top surface was leveled. The clay was allowed to dry slowly, after which cracks were filled with a clay paste and the surfaces were then coated with fresh cow-dung paste. The use of cow-dung wash over the floor and the mud walls is a common practice in rural India. The clay soil appeared to differ greatly in color and physical properties from the type of mud that is generally pre-

[1] This work was conducted under funds allotted by the National Military Establishment to the Bureau of Entomology and Plant Quarantine.

[2] Now with the Regional Malaria Organization, Coimbatore, South India.

ferred and available in India. These differences were overlooked because of the practical difficulties of obtaining materials identical with those used in India.

Treatment of Panels.—All three types of surfaces were treated with DDT in a kerosene solution; an emulsion prepared from a concentrate containing 25 per cent of DDT, 65 per cent of xylene, and 10 per cent of Triton X-100 (monoisooctyl phenyl polyethylene glycol ether); and a suspension prepared from a concentrate containing 10 per cent of DDT, 80 per cent of Cellosolve (ethyl ether of ethylene glycol), and 10 per cent of Nopco 1216 (sulfonated sperm oil). In a second series of tests using only clay panels, the emulsion and the suspension were compared with an emulsion pre-

TABLE 1

Number of weeks various DDT formulations applied to different surfaces caused 90 to 100 per cent mortality of house flies and mosquitoes after two-hour exposures

(All treatments made with 1 per cent DDT solutions, emulsions, or suspensions)

SURFACE	DDT FORMULATIONS	FLIES	MOSQUITOES
Straw	Kerosene solution	28	24
	Xylene-Triton X-100 emulsion	28	24
	Cellosolve-Nopco 1216 suspension	32	32
Palm	Kerosene solution	1	1
	Xylene-Triton X-100 emulsion	4	4
	Cellosolve-Nopco 1216 suspension	24	24
Clay	Kerosene solution	0-0*	0-0
	Xylene-Triton X-100 emulsion	0-0*	1-1
	Cellosolve-Nopco 1216 suspension	1-0*	4-0
	Shell E-407-R-soap emulsion	1	1
	Suspension of wettable powder†	1	1
	Suspension of wettable powder B	4	4
	Colloidal suspension	1	1

* Figures shown are for two series of tests.

† Differences in chemical composition of the two materials were not determined.

pared from a concentrate containing 65 per cent of Shell E-407-R[3] solvent, 10 per cent of a 20-per cent soap solution, and 25 per cent of DDT, two commercial 50 per cent wettable powders (A and B), and a proprietary colloidal suspension. All finished sprays contained 1 per cent of DDT. A small electric sprayer with a DeVilbiss nozzle was used to apply these treatments at the rate of 200 mg. of DDT per square foot. The treatments were applied to duplicate panels of each type, and two other panels of each type were left untreated to serve as controls.

Test Procedure.—Laboratory-reared 5-day-old house flies (*Musca domestica* L.) and 4-day-old mosquitoes (*Anopheles quadrimaculatus* Say) were used as test insects. All the tests were conducted at room temperatures ranging from 80° to 85° F. At intervals during a 40-week period in the first series of tests and during 12 to 16 weeks

[3] An aromatic petroleum solvent said to be equivalent to the Assam Oil Company's medium kerosene extractive.

in the second series, approximately 20 to 40 insects under half-petri dishes were exposed to the surfaces for two hours. After the exposure period the insects were anesthetized with carbon dioxide and transferred to clean holding cages. A piece of cotton dipped in honey-water was placed on top of the cages to provide food for the insects that survived exposure to DDT. Mortality counts were made 24 hours after treatment.

Results.—In the tests on straw panels (table 1), the kerosene solution and the emulsion formulations caused mortalities of 90 to 100 per cent of the mosquitoes for 24 weeks and of flies for 28 weeks. The suspension retained a high degree of effectiveness against both species for 32 weeks. On the palm panels the treatment applied as a kerosene solution was effective the first week after treatment but not the fourth, the emulsion treatment was effective for four weeks but not for eight, and the suspension treatment continued to kill more than 90 per cent of both insects for 24 weeks.

On clay panels one commercial sample of wettable powder was the best of the treatments, but remained effective for only 4 weeks. The kerosene solution failed to give 90 per cent mortality the first week and the xylene-Triton emulsion was effective the first week against mosquitoes but not flies. Another emulsion (prepared with Shell E-407-R and soap) was effective against both species for one week.

Summary.—Laboratory tests were carried out to determine the relative effectiveness against house flies and mosquitoes of different DDT formulations when applied as residual treatments to three types of wall surfaces commonly used in the construction of houses in rural India—straw and palm-leaf thatches, and clay with a surface wash of cow dung. Adults of *Musca domestica* L. and *Anopheles quadrimaculatus* Say were employed as test insects. On each type of surface suspensions of DDT were more effective than solutions or emulsions, although on straw surfaces the last two were effective for nearly as long as the suspensions. On clay panels the best of the treatments, a commercial wettable powder, was effective for only four weeks.

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PRELIMINARY FIELD STUDIES ON THE USE OF HEAVY DOSAGES OF DDT AND BENZENE HEXACHLORIDE AS RESIDUAL MOSQUITO LARVICIDES

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Studies were initiated in the spring of 1948 to develop a mosquito larviciding treatment that would have an extended residual larvicidal action. Until the introduction of several new insecticides during and since World War II, the methods and materials employed for the chemical control of mosquito larvae had not changed appreciably for many years. Oils were used as larvicides as early as 1892 (Howard, 1892). Until recently the most commonly used materials were paris green as a dust and No. 2 fuel oil or waste oil as a spray. Both materials gave control of anopheline larvae, and the oils also controlled culicine larvae, but with the type of equipment and amounts of material used, the labor cost was high, especially with the oil sprays.

After the discovery of the effectiveness of DDT (2,2-bis(para-chlorophenyl)-1,1,1-trichloroethane) as a mosquito larvicide, the method of application and amounts of material used underwent radical changes (Ferguson et al., 1947). Low-volume formulations were introduced, and by using the wind to carry the finely atomized spray, treatments could be made with a large saving in manpower as compared with former methods (Mathis et al., 1947). Many formulations and materials have been used, with most of the emphasis in the earlier work being placed on the use of DDT. The earliest work indicated that while this material was quite effective with dosages as low as 0.1 pound per acre, it was also toxic to other aquatic organisms, especially when used as an emulsion (Arnold et al., 1945). Consequently, the main effort in the past few years has been directed at finding a formulation that would give satisfactory control of mosquito larvae, yet not be harmful to other aquatic organisms. The formulation of 0.05 pound of DDT in 1 gallon of No. 2 fuel oil with 0.5 per cent Triton-B1956[2] applied at the rate of 1 gallon of finished spray per acre (Ferguson et al., 1947), as presently recommended by the U. S. Public Health Service, has proved satisfactory for the control of anopheline larvae in the southern United States, without causing significant damage to wildlife (Tarzwell 1948). Routine applications of DDT larvicide by airplane at discharge rates of 0.1 pound of DDT per acre have been found to have no significant harmful effect on wildlife during 2 years of treatment (Tarzwell, 1948).

Even with all the advantages of the new methods of larviciding, the ultimate goal of developing a larvicide that would effect control for several weeks was not obtained, as these treatments, like former methods, must be repeated at approximately weekly

[1] From Technical Development Division, Savannah, Ga.

[2] An emulsifying-spreading agent produced by Rohm and Haas Company, Philadelphia, Pa. This does not represent an andorsement of the product by the Public Health Service.

intervals in most cases. Due to the increased attention given to culicine mosquitoes as potential disease vectors, more emphasis has been placed recently on control measures for culicine larvae. Some indications have been obtained that certain species of culicine larvae are more difficult to control than anopheline larvae (Eide et al., 1945). These factors, together with repeated requests for a larvicide that would give a long residual effect in places that have no wildlife value, led to the work included in this paper. *Pending further investigations none of the formulation mentioned herein should be used where fish or other aquatic wildlife are of any importance.*

PROCEDURE

All work reported herein was done in the Camp Stewart Military Reservation located near Savannah, Ga. This area, with its numerous cypress and black-gum swamps, is typical of the southeastern coastal sections of the United States. The smaller, land-locked swamps usually become dry at some period during each year, so that the fish and many other aquatic orgnisms in them die. These small, land-locked ponds, together with borrow pits, were selected for treatment. The size of the plots ranged from approximately 0.1 to 2 acres. The emergent vegetation varied from only marginal to a cover of 75 per cent. Most of the ponds were very rich in organic material, and since it has been shown that mud containing large quantities of organic matter inactivates certain amounts of DDT (Upholt, 1947), it was believed that it would be relatively difficult to establish a long period of effective control in this type of location. These test ponds were not subject to strong wind and wave action, or to continuous exchange of water by influent and effluent flows.

Treatments were applied at irregular intervals from April through August, 1948 and observations on mosquito larval development in the test ponds were continued until November, when untreated ponds stopped producing mosquitoes. The various treatments employed were tested concurrently during this period.

A type of selective sampling was employed which was restricted to those portions of each pond that contained vegetation, where past experience had shown that mosquito larvae would be found if they were present. Samples were taken with dippers at approximately uniform distances throughout the favorable portions of each pond. Approximately the same number of samples was taken in each pond each time it was inspected, unless a change in the size of the pond due to flooding or drying or an increase in the amount of vegetation present in the pond, made it necessary to increase or decrease the number of samples required for an adequate inspection. Every effort was made to obtain a thorough coverage of the pond at each inspection. All ponds were sampled before treatment, 24 hours afterward, and at weekly intervals thereafter as long as the treatments were considered to be effective. The mosquito larvae were classified as small or large, anopheline or culicine, and a collection of fourth instar culicine larvae was made throughout the tests.

In previous studies, the degree of control obtained was expressed in terms of percentage reduction of mosquito larvae in the test plots, based on the pretreatment and posttreatment larval populations. This method was considered adequate when inspections were made 24 hours after treatment, and perhaps for inspections made a week later. However, since some tests included in this report covered periods as long

as 4 months, this former method of evaluation was considered inadequate, due to changes in the intensity of mosquito breeding which could possibly occur during this interval.

In the work reported herein, no attempt was made to measure the *degree* of control by comparison of populations. No comparisons were made between populations in different ponds, nor between pretreatment and posttreatment populations in the same pond. Comparisons were made only on the week to week *development* of mosquito larvae in the same pond after treatment. The initial larval population in each pond was practically eliminated by each of the formulations tested. Later on, many inspections were made in which eggs and small larvae were found, yet the following week none were found, or those found were small. Conclusions as to the period of effective control of each treatment were based on this over-all picture of larval development in each pond separately after treatment. A treatment was considered to have become ineffective when any weekly inspection revealed the presence of one or more large larvae, supported by an increase in the stage of development and distribution of the small larvae present, as compared to the previous weekly inspection in that particular pond. In effect, the degree of control obtained was approximately 100 per cent during the periods when the test ponds were considered to be under effective control, for when sampling indicated that larvae were beginning to develop to maturity in a treated pond, the treatment in that pond was immediately considered to have become ineffective, regardless of how the larval population in the pond at that time might compare with pretreatment populations in the same pond or with populations in other ponds.

Although no routine inspections were made in untreated check ponds concurrently with those made in the treated ponds, sufficient observations were made at irregular intervals in untreated ponds to assure that mosquito larval development was in progress continuously throughout the test period.

Gross observations were made on the condition of other aquatic organisms at each examination since, from previous work with lower dosages (Tarzwell, 1948), it was expected that these heavier treatments with DDT would destroy fish and most other forms of aquatic life. Little information is available on the toxicity of benzene hexachloride to aquatic organisms, but preliminary reports from the U. S. Fish and Wildlife Service indicate that benzene hexachloride is less toxic to fish than is DDT (Linduska and Surber, 1948).

EQUIPMENT AND OPERATIONS

The equipment employed was the same hand-operated type as that used in applying the low-volume, surface-film applications, with the exception of the nozzle used to apply the larger volumes of spray. A detailed discussion has been given of this type of equipment in a previous publication (Ferguson et al., 1947).

Spraying operations also were quite similar to those of the low-volume, surface-film treatments. Most treatments were made with 1 or 2 gallons of finished spray per acre, and these were made using the 30-foot swath width, and 75-feet-per-minute pace. In such applications the wind was depended upon to carry the finely atomized spray. A few treatments were made in which 5 or 15 gallons of spray per acre were

used. In applying these latter treatments the entire water surface was sprayed with a No. 5002 Spraying Systems nozzle which produced a flat, fan-shaped spray pattern. Stock concentrates were made in the laboratory and, after plots were selected, the amounts of the concentrate and water necessary for treatment were computed. The water to be used was taken from the test ponds and strained through a cloth to keep out trash that would clog the nozzle. With the benzene-hexachloride water wettable powder, a sufficient amount was weighed, and the water added at the plot to be sprayed.

FORMULATIONS TESTED

One requirement of the solvents to be used in these tests was that they be able to dissolve sufficient amounts of DDT or benzene hexachloride so that the volume of finished spray would be 1 or 2 gallons per acre. Another requirement was that the concentrate have a specific gravity as near that of water as possible, on the theory that if the concentrate had approximately the same specific gravity as water, it would reduce the tendency of the emulsion to separate after spraying. With these requirements as the only basis for selecting the solvent, the following concentrates were made and tested:

(1) DDT, tech. grade	3 pounds
Triton X155[3]	379 ml.
PD544C[4]	to make 1 gallon
(2) DDT, tech. grade	3 pounds
Triton X155	189 ml.
PD544C	to make 1 gallon
(3) DDT, tech. grade	3 pounds
Triton X155	379 ml.
Xylene	to make 1 gallon
(4) DDT, tech. grade	1 pound
Triton X155	189 ml.
Fuel oil, No. 2	to make 1 gallon
(5) Benzene Hexachloride, tech. grade containing 10 per cent gamma isomer	1 pound
Triton X155	189 ml.
Xylene	to make 1 gallon
(6) Benzene Hexachloride, water wettable powder containing 25 per cent gamma isomer	4 pounds
Water	to make 5 gallons

From these DDT stock concentrates other formulations were prepared. Preliminary tests were made with each formulation at the beginning of the season to determine if

[3] An emulsifier produced by the Rohm and Haas Company, Philadelphia, Pa.

[4] A solvent produced by the Socony-Vacuum Oil Company, New York, N. Y. This does not represent an endorsement of the products by the Public Health Service.

varying the amount of emulsifier or volume of finished spray influenced the results obtained. These were made using dosages of 1 and 3 pounds of DDT per acre. The amount of emulsifier used was 5 and 10 per cent of the original stock concentrates to determine if these higher quantities of emulsifier would aid in keeping the insecticide in an emulsified form in the pond waters. In some tests the emulsions were prepared by adding 14 parts of water to 1 part concentrate, and were applied at the rate of 15 gallons of finished spray per acre. In others, the 3-pound-per-acre formulation was prepared by mixing one part of concentrate number 1, 2, or 3 with one part water and the finished emulsion was applied at the rate of 2 gallons per cacre. The 1-pound-per-acre formulation was prepared by mixing $\frac{1}{3}$ part of the stock concentrate number 1, 2, or 3 with $\frac{2}{3}$ part water, and the resulting emulsion was applied at the rate of 1 gallon per acre. The formulation in which fuel oil was the solvent was applied as an emulsion made by adding 1 part water for each part of concentrate and applying at the rate of 2 gallons of finished spray per acre. It was applied also in the concentrate form as a surface film.

In these first few preliminary tests, no difference could be detected between tests in which different amounts of emulsifier or volume of spray were used. In the tests with the 1-pound-of-DDT-per acre formulations, however, those in which PD544C were used as the solvent appeared to give slightly better results than those in which fuel oil or xylene was the solvent. Therefore, for the remainder of the season the amount of emulsifier in all formulations was reduced to 5 per cent; PD544C was used as the solvent in all formulations; and the volume of finished spray used was 1 and 2 gallons per acre for the 1- and 3-pounds-of-DDT-per-acre formulations, respectively.

No variations were made in the benzene hexachloride emulsion formulation, only 1 pound of technical grade benzene hexachloride (10 per cent gamma isomer) in xylene with 5 per cent emulsifier, being tested. All tests were made with emulsions prepared by mixing one part stock concentrate with one part water and applied at the rate of 2 gallons of finished spray per acre. The wettable powder was also used at only one concentration.

RESULTS

Since no differences could be detected in the length of the period of effective control obtained with dosages of the same amount of DDT but with different amounts of emulsifier or water added to the finished spray, all treatments containing the same amount of DDT in the same solvent were considered as a unit in drawing conclusions regardless of the amount of emulsifier or spray volume used.

Seven treatments were made with the DDT-PD544C formulations applied at the rate of 3 pounds of DDT per acre. Due to drying or flooding of some test plots to such an extent that further inspections were of no value, some tests were begun which were not completed. Only 4 plots remained available for further observations after the 8th week, one of which was considered to be ineffective after that time. Another of these plots was dry at the 4th week, but had refilled by the 6th week and was considered to be giving control up to the 15th week, when it was dry again. Of the remaining two plots, one had given control for 12 weeks and the other for 16 weeks, and both were still effective when observations were discontinued with the advent of cold weather. A summary of the larval counts in these tests is given in table 1.

Six tests were made with the DDT-PD544C formulation applied at the rate of 1 pound of DDT per acre. When all were completed, the trend was found to be very

TABLE 1

Summary of larval counts from six treatments with emulsion sprays containing 3 pounds of DDT in PD544C per acre

INSPECTION INTERVALS	TOTAL NUMBER OF DIPS	TOTAL NUMBER OF LARVAE FOUND				NUMBER PLOTS INCLUDED
		Culicines		Anophelines		
		Small	Large	Small	Large	
Pretreatment	264	479	90	316	49	6
24 hours	271	0	0	0	4	6
1 week	283	0	0	2	0	6
2 weeks	258	4	0	1	0	6
3 weeks	247	51	0	0	0	6
4 weeks	145	9	0	3	0	4
5 weeks	122	17	0	1	2	3
6 weeks	229	17	0	9	0	6
7 weeks	161	26	0	4	0	5
8 weeks	98	0	0	4	2	3
9 weeks	119	10	0	1	0	3
10 weeks	115	3	1	18	0	3
11 weeks	119	6	1	7	0	3
12 weeks	82	0	0	2	1	3
13 weeks	36	0	0	3	0	1
14 weeks	71	3	0	12	1	2
15 weeks	30	0	0	0	0	1
16 weeks	20	0	0	0	0	1

Note: One plot treated was not included in the table as it had increased in size by 2nd week and by 4th week it was 3 times its original size as a result of flooding. On the 3rd week, large larvae were found in the flooded marginal areas of this pond.

TABLE 2

Summary of larval counts from six treatments with emulsion sprays containing 1 pound of DDT per acre in PD544C

INSPECTION INTERVALS	TOTAL NUMBER OF DIPS	TOTAL NUMBER OF LARVAE FOUND				NUMBER PLOTS INCLUDED
		Culicines		Anophelines		
		Small	Large	Small	Large	
Pretreatment	305	1218	205	493	86	6
24 hours	299	0	0	0	3	6
1 week	326	5	0	0	0	6
2 weeks	319	20	0	23	3	6
3 weeks	302	86	1	76	4	6
4 weeks	141	10	0	153	9	3
5 weeks	56	37	0	45	9	1

consistent. In most instances small larvae were found 2 weeks after treatment in small numbers and increased each week thereafter. In one test, 3 large and several

small anopheline larvae were found the 2nd week after treatment, but the treatment was still considered effective because all larvae were obtained in one isolated pocket, indicating that the plot was not breeding generally. Three of these treatments gave

TABLE 3

*Summary of larval counts from five treatments with emulsion sprays containing 1 pound of technical benzene hexachloride per acre**

INSPECTION INTERVALS	TOTAL NUMBER OF DIPS	TOTAL NUMBER OF LARVAE FOUND				NUMBER PLOTS INCLUDED
		Culicines		Anophelines		
		Small	Large	Small	Large	
Pretreatment	271	134	46	281	29	5
24 hours	241	0	2	2	0	5
1 week	208	3	0	0	0	5
2 weeks	143	0	0	2	0	3
3 weeks	239	17	0	17	0	5
4 weeks	239	48	1	74	0	5
5 weeks	175	91	2	154	6	4
6 weeks	45	11	0	73	1	1

* Approximately 10 per cent gamma isomer; solvent was xylene.

TABLE 4

Summary of larval counts from three treatments with 4 pounds of benzene hexachloride wettable powder per acre applied as a spray*

INSPECTION INTERVALS	TOTAL NUMBER OF DIPS	TOTAL NUMBER OF LARVAE FOUND				NUMBER PLOTS INCLUDED
		Culicines		Anophelines		
		Small	Large	Small	Large	
Pretreatment	91	186	25	137	20	3
24 hours	102	1	0	0	0	3
1 week	108	0	0	0	0	3
2 weeks	96	0	0	0	0	3
3 weeks	64	0	0	0	0	2
4 weeks	110	1	0	0	0	3
5 weeks	111	28	0	3	0	3
6 weeks	110	3	1	8	0	3
7 weeks	109	33	0	23	0	3
8 weeks	104	96	4	80	5	3
9 weeks	76	6	0	10	0	2
10 weeks	73	3	0	39	1	2
11 weeks	21	0	0	16	0	1

* 25 per cent gamma isomer.

adequate control for 3 week, two for 4 weeks, and one for 5 weeks. A summary of the larval counts in the tests with 1 pound of DDT in PD544C per acre is given in table 2.

With xylene or fuel oil as the solvent, 1 pound of DDT per acre applied as an emulsion gave effective control for only 2 weeks. A similar dosage in fuel oil applied as a surface film at the rate of 1 gallon per acre also gave effective control for only 2 weeks.

Seven tests were made with the technical benzene hexachloride formulation. In two of the test plots, which flooded during the first week after treatment, control was effective for only 3 weeks, and these two plots are not included in table 3 due to these abnormal conditions. One pond was still under effective control at the end of 4 weeks when it became dry. Of the remaining four plots, three gave effective control for 5 weeks and one for 6 weeks.

Only 3 tests were made with the 25-percent-gamma-isomer benzene-hexachloride wettable powder. In one test, which involved a population of anopheline larvae only, no larvae were found until the 7th week after treatment. The first large larvae were found on the 10th week and the treatment was not considered to be effective after this time. Another plot, which contained both anopheline and culicine larvae remained under control for 8 weeks. In this plot the water was higher than pretreatment level on the 3rd week and continued to rise until, by the 5th week, it was flooded. The influence of this flooding is not known. The same plot was retreated and was giving control through the 11th week when inspections were discontinued because of unfavorable breeding conditions. Table 4 gives a summary of the larval counts in these tests.

Identification of the fourth instar culicine larvae collected in the test pond throughout the season revealed that the following species were present: *Culex apicalis*, *C. erraticus*, *C. salinarius*, *C. pilosus*, *C. restuans*, *Culiseta melanura*, *Uranotaenia sapphirina* and *Aedes dupreei*. Of the species collected, *C. apicalis*, *C. erraticus* and *U. sapphirina* were the most numerous.

DISCUSSION

A review of the data in tables 1 to 4 and the previous discussion presented on procedure will demonstrate that a conservative attitude has been taken in concluding when a treatment ceased to be effective. The number of larvae being recovered in the treated plots at the time most of the treatments were considered to have become ineffective was seldom more than a small fraction of the original pretreatment population. This reduction may have been due in some instances to reduced mosquito breeding in general, but in others appreciable control was undoubtedly still being obtained. The periods of effective control shown for these treatments are considered to be the minimum periods, and in actual field operations significant reduction in mosquito breeding would probably extend over considerably longer periods.

When a plot that had been treated with 3 pounds of DDT per acre had increased in size over the pretreatment level 1 week after treatment, it was noticed that the larval infestation began building up immediately in the outer, newly-flooded margins. In a plot that became dry 4 weeks after treatment and had refilled by the 6th week there was no larval development until the 12th week, while an untreated pond a few feet away continued breeding. In many ponds it was noticed that the general insect population returned much more rapidly to the open-water areas in the center of the ponds than to the margins where treated vegetation was present. These phenomena indicated that the vegetation and debris, rather than the water may be retaining the toxic agent. To test this possibility, vegetation from treated ponds was brought into the laboratory. Chemical analyses indicated that DDT was present on vegetation from a pond treated 11 weeks previously. Vegetation from treated ponds, when added

to dishes containing fourth instar larvae of laboratory-reared *Anopheles quadrimaculatus*, produced approximately 70 per cent larval mortality within 24 hours. On the basis of these laboratory results and field observations, it seems resaonable to conclude that the deposition of DDT on the vegetation and debris of the ponds was largely responsible for the long duration of effectiveness of the treatments.

Observations on the Effects of Treatments on Other Aquatic Organisms

Gross observations were made on other aquatic organisms found in the test plots before and after treatment. No special effort was made to find any of these organisms, only those observed or picked up in the dipping process being included.

In ponds which received 3 pounds of DDT per acre, very few organisms were noticed the first week after treatment except for small nymphs of dragon flies and damsel flies. Gyrinidae, and occasionally adult forms of other beetles were noted, but these probably came in after treatment. Very few living fish were noticed after treatment, while many were found dead immediately following the treatment. In ponds that flooded after treatment, and into which fish apparently came during the flooding, the fish seemed to be able to survive.

With treatments of 1 pound of DDT per acre, the results followed a similar trend but the reduction was not so drastic; and by the 4th week, most forms of insect life had returned to the ponds. Although considerable numbers of dead fish were found immediately after treatment, some apparently survived.

In plots treated at the rate of 1 pound of technical benzene hexachloride per acre, the insect population was noticeably reduced; but one of the most striking observations was that no dead fish were found. Two plots received two treatments each, and apparently the fish still were normal.

With the 25 per cent gamma isomer of benzene-hexachloride wettable powder, most insect life was destroyed and was slow in returning. Many fish were killed, but some sunfish and many minnows survived.

No observations were made of any adverse effects of these treatments on terrestrial forms of wildlife, or on domestic animals which might have used the test ponds as a source of drinking water.

SUMMARY

DDT and benzene hexachloride, when used at heavy dosages in small land-locked ponds in an effort to establish a long period of effective control against mosquito larvae, gave the following results against both anopheline and culicine larvae:

1. DDT when applied as an emulsion at the rate of 3 pounds per acre gave control for from 8 to 16 weeks.

2. One pound of DDT per acre applied as an emulsion gave from 3 to 5 weeks of control.

3. Benzene hexachloride (10 per cent gamma isomer) applied as an emulsion at the rate of 1 pound per acre gave 5 weeks of control.

4. A 25 per cent gamma isomer wettable powder of benzene hexachloride gave from 8 to 11 weeks of control when applied in spray form at the rate of 4 pounds of the powder per acre.

5. Aquatic insects were greatly reduced by all of the treatments.

6. Fish were practically eliminated by the treatment with 3 pounds of DDT per acre, were greatly reduced by the application of 1 pound of DDT per acre, and apparently suffered no kill with two repeated applications of 1 pound of technical benzene hexachloride (10 per cent gamma isomer) per acre. Some survived treatment with the 25 per cent gamma-isomer-benzene-hexachloride water wettable powder at the rate of 4 pounds of powder per acre. *The DDT treatments should not be used where fish or wildlife are of any significance. Pending further investigation benzene hexachloride also should be used with extreme caution.*

7. Results from field tests and observations from biological and chemical laboratory tests indicate that the long period of effective control obtained in the tests with 3 pounds of DDT per acre resulted largely from deposits of DDT adhering to the vegetation and debris in the ponds.

8. The efficiency of the treatments was lost if the ponds increased in size during the test period over their size at the time of treatment.

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EFFECTS OF X-IRRADIATION ON *PLASMODIUM GALLINACEUM* AND *PLASMODIUM LOPHURAE* INFECTIONS IN YOUNG CHICKS

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In 1944 Naiman reviewed the limited literature on the effect of X-irradiation on the resistance of experimental animals to various infections and reported that the severity of the infection was increased in rats infected with *Trypanosoma lewisi*.

The effect of X-rays on malarial parasites has recently been studied by several investigators and among these two have dealt with the effect of irradiation on the parasitemia. Rigdon and Rudisell (1945) studied *Plasmodium lophurae* in young chickens and found that the irradiated birds had lower parasite counts than the unirradiated controls. Taliaferro *et al.*, (1945) on the other hand, working with *Plasmodium gallinaceum* and *P. lophurae* in chickens found that irradiation of the host resulted in increased parasitemia.

The present paper supports the findings of Taliaferro *et al.* and includes data to show the effects of quinine treatment and sex of the host on the parasitemia of irradiated chicks.

MATERIALS AND METHODS

Two different strains of malaria parasites were used: the 8A strain of *Plasmodium gallinaceum* and a strain of *P. lophurae* obtained from Dr. L. A. Terzian of the Naval Medical Research Institute.

The same strain of Rhode Island Red chicks (*Gallus gallus*) was used throughout. The young birds were delivered to the laboratory when one day old and were infected when 4 to 12 days old; 70 were four days, 81 were six days, 298 were seven days and 10 were ten days old when inoculated. The study involved a total of 459 birds.

Chicks with blood-induced infections were used as donors when 20 to 50 per cent of the red blood cells contained parasites. Blood was drawn from the heart, heparinized and diluted with 0.85 per cent NaCl solution to give a suspension containing the desired concentration of parasitized erythrocytes. The young birds were infected by injecting 0.1 to 0.2 cc. of the diluted parasitized blood directly into the right jugular vein.

Smears of the peripheral blood were made each morning, usually beginning on the day following inoculation. They were air dried and stained with Giemsa stain after fixation with methyl alcohol. In positive smears the percentage of red blood cells parasitized was determined by counting the number infected per 10,000 red blood cells. The sample counted was such as to give a probable error of 10 per cent or less in counts above 30 per 10,000 red blood cells.

The sex of the chicks was determined by examination of the gonads at autopsy.

Selected groups of chicks were exposed to single beam roentgen irradiation while covered with two layers of cheesecloth. The X-ray tube was operated at 200 kilovolts, 20 milliamperes with 0.25 mm. copper and 1.06 mm. aluminum filters. The distance from the center of the target to the approximate center of the group of chicks was 40 cm. The intensity of irradiation was 68 roentgens per minute as measured in air with a Victoreen chamber at 40 cm.

Birds treated with quinine were given the drug twice daily for 4, 8 or 11 days. The first dose of drug was given about five hours before inoculation of parasitized blood. The dosage of the drug was 0.2 mgm./gm. body weight, the least amount which had previously been found (Coatney and Sebrell, 1946) to reduce the parasitemia of treated birds to 25 per cent or less of that of the controls.

EXPERIMENTAL RESULTS

Plasmodium gallinaceum infections in irradiated chicks. The first experiment was designed to determine the effect of a given amount of irradiation (400 r) when administered at various times in relation to infection with 10^5 red blood cells parasitized with *P. gallinaceum*. Forty seven-day old chicks were divided into 4 groups of 10 birds each: group A was irradiated 4 days before, group B the day of, and group C 2 days after infection. Group D served as the unirradiated control.

As shown in Table 1 and Figure 1 the peak parasitemia was higher in the treated chicks than in the controls and those in group B, irradiated on the day of inoculation, showed a higher parasitemia than either of the other treated groups.

Inasmuch as this initial experiment indicated a greater effect on the parasitemia when the irradiation was given on the day of inoculation, it was decided to administer succeeding treatments at that time.

Experiment II included 40 seven-day old chicks each of which was inoculated with 16×10^6 parasitized red blood cells. Twenty of these birds received 400 r on the day of inoculation and 20 were untreated controls. Experiment III included 22 six-day old chicks. Each was inoculated with 16×10^6 parasitized red blood cells. Eleven birds received 400 r on the day of inoculation and 11 were untreated controls. Experiment IV included 38 seven-day old birds each of which received 10^7 parasitized red blood cells. Twenty were exposed to 400 r on the day of infection and 18 were kept as untreated controls. Experiment V included 59 birds six days old at the time of inoculation with 16×10^6 parasitized red blood cells. Twenty-nine of these birds were exposed to 400 r and 30 were untreated controls.

In each instance, as shown in Table 1 and Figure 1, the irradiated birds showed significantly higher parasite counts than the untreated controls.

P. lophurae infections in irradiated chicks. It is not uncommon for chicks infected with *P. gallinaceum* to show parasitization of 80 per cent or more of the circulating red cells so there is little range for an exhibition of greater parasitemia. In an attempt to find a better host-parasite model for the observation of changes in parasitemia following X-irradiation it was decided to carry out studies using *P. lophurae* since infections with this parasite seldom involve more than 60 per cent of the circulating red cells.

Experiment VI included ten 10-day old chicks each of which was inoculated with 30×10^6 erthyrocytes infected with *P. lophurae.* Five birds received 400 r four days

TABLE 1

The effect of X-irradiation on the parasitemia in young chicks infected with Plasmodium gallinaceum

EXP.	GROUP	NO. INOCULATED	AGE AT INOCULATION	PARASITIZED rbc INOCULATED	DOSAGE AND TIME OF X-RAY*	MEAN PER CENT ERYTHROCYTES PARASITIZED ON DAY						
						3	4	5	6	7	8	9
			days									
I†	A	10	7	10^5	400r (d − 4)			17.1	32.4	63.6	73.9	69.4
	B	10	7	10^5	400r (d)			18.5	41.5	77.1	83.8	74.5
	C	10	7	10^5	400r (d + 2)			12.8	28.0	59.1	68.2	80.2
	D	10	7	10^5	none			10.4	23.1	57.9	63.8	58.0
	$\frac{D}{SE_D}$‡ Group (B vs D)							2.45	3.18	2.11	3.88	N.S.
II	A	20	7	16×10^6	400r (d)	22.7	70.9	84.1	69.8			
	B	20	7	16×10^6	none	20.3	59.1	82.4	73.8			
	$\frac{D}{SE_D}$					N.S.	2.30	N.S.	N.S.			
III	A	11	6	16×10^6	400r (d)	24.3	74.7	89.0				
	B	11	6	16×10^6	none	17.4	45.0	73.8				
	$\frac{D}{SE_D}$					1.96	5.42	3.24				
IV	A	20	7	10^7	400r (d)	19.8	71.9	70.1	67.7	72.7		
	B	18	7	10^7	none	21.2	58.1	64.8	64.7	56.5		
	$\frac{D}{SE_D}$					N.S.	2.08	N.S.	N.S.	3.39		
V	A	29	6	16×10^6	400r (d)	20.6	58.1	75.2	64.0			
	B	30	6	16×10^6	none	15.7	44.4	70.8	60.8			
	$\frac{D}{SE_D}$					2.46	3.77	2.37	N.S.			

* "r" denotes roentgens and "d" the day of inoculation.

† Some 5th day counts in this experiment were reported as "less than 6%." For purposes of calculating averages and statistical significance, these counts were taken as 6%.

‡ $\frac{D}{SE_D}$ represents the observed difference divided by its Standard Error. Values greater than 2.0 are considered significant. N.S. indicates that the observed difference was not statistically significant.

before infection and five were kept as untreated controls. Experiment VII included 35 chicks each four days old at the time of inoculation with 60×10^6 infected erythrocytes. Seventeen of these birds received 400 r on the day of inoculation and 18 were

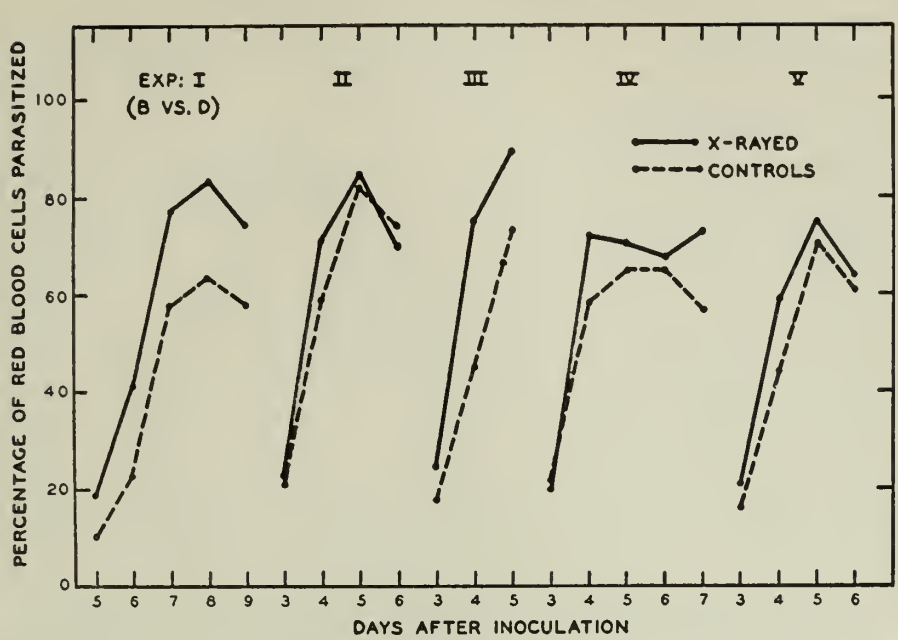

FIG. 1. Average daily parasite counts in irradiated and control chicks infected with *Plasmodium gallinaceum*.

TABLE 2

The effect of X-irradiation on the parasitemia in young chicks infected with Plasmodium lophurae

EXP.	GROUP	NO. INOCULATED	AGE AT INOCULATION	PARASITIZED rbc INOCULATED	DOSAGE AND TIME OF X-RAY*	MEAN PER CENT ERYTHROCYTES PARASITIZED ON DAY						
						3	4	5	6	7	8	9
			days									
VI	A	5	10	30×10^6	400r (d − 4)		12.8	27.3	50.4	59.2	48.0	27.8
	B	5	10	30×10^6	none		12.6	21.4	37.0	46.2	44.6	36.0
	$\frac{D}{SE_D}$†						N.S.	N.S.	N.S.	2.14	N.S.	N.S.
VII	A	17	4	60×10^6	400r (d)	12.8	28.9	61.5	80.2	68.1	58.1	
	B	18	4	60×10^6	none	11.3	21.5	48.6	59.4	61.6	49.0	
	$\frac{D}{SE_D}$					N.S.	3.12	2.61	4.24	N.S.	N.S.	
VIII	A	15	4	57×10^6	400r (d − 1)	11.6	19.8	54.9	71.3			
	B	20	4	57×10^6	none	11.7	21.2	44.2	55.9			
	$\frac{D}{SE_D}$					N.S.	N.S.	2.04	2.34			

* "r" denotes roentgens and "d" the day of inoculation.

† $\frac{D}{SE_D}$ represents the observed difference divided by its Standard Error. Values greater than 2.0 are considered significant. N.S. indicates that the observed difference was not statistically significant.

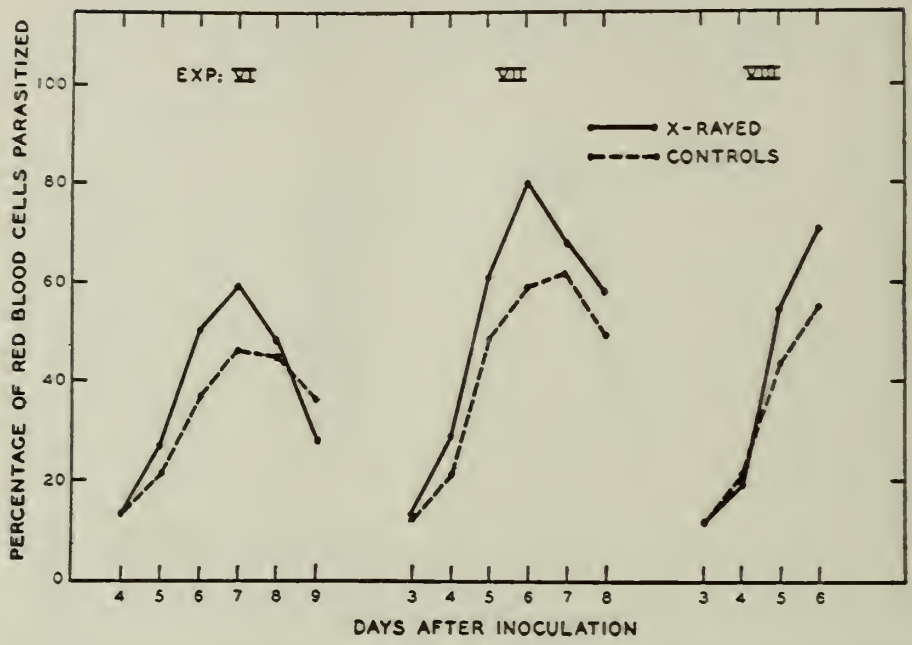

FIG. 2. Average daily parasite counts in irradiated and control chicks infected with *Plasmodium lophurae*.

TABLE 3

The effect of X-irradiation on parasitemia in young chicks infected with Plasmodium gallinaceum *and treated with quinine*

EXP.	GROUP	NO. INOCULATED	AGE AT INOCULATION	PARASITIZED rbc INOCULATED	DOSAGE AND TIME OF X-RAY*	DURATION OF QUININE TREATMENT	MEAN PER CENT ERYTHROCYTES PARASITIZED ON DAY								
							2	3	4	5	6	7	8	9	10
			days			*days*									
IX	A	10	7	16×10^6	400r (d-1)	4	2.1	18.2	31.6	53.6	68.0	60.0			
	B	10	7	16×10^6	none	4	.4	6.3	18.7	39.1	53.4	55.9			
	$\frac{D}{SE_D}$†						3.39	2.43	N.S.	N.S.	N.S.	N.S.			
X	A	15	7	16×10^6	400r (d-1)	8		3.1	7.4	17.3	24.0	16.6	19.9		
	B	19	7	16×10^6	none	8		.9	1.6	4.7	9.6	10.2	10.6		
	$\frac{D}{SE_D}$							N.S.	N.S.	N.S.	N.S.	N.S.	N.S.		
XI	A	19	7	10^7	400r (d)	11	.9	6.4	15.1	28.2	38.3	43.2	33.9	27.9	32.6
	B	17	7	10^7	none	11	.5	2.5	6.1	12.9	27.3	23.2	12.6	10.5	12.5
	$\frac{D}{SE_D}$						2.3	2.35	2.26	2.14	N.S.	2.24	3.20	2.75	3.92

* "r" denotes roentgens and "d" the day of inoculation.

† $\frac{D}{SE_D}$ represents the observed difference divided by its Standard Error. Values greater than 2.0 are considered significant. N.S. indicates that the observed difference was not statistically significant.

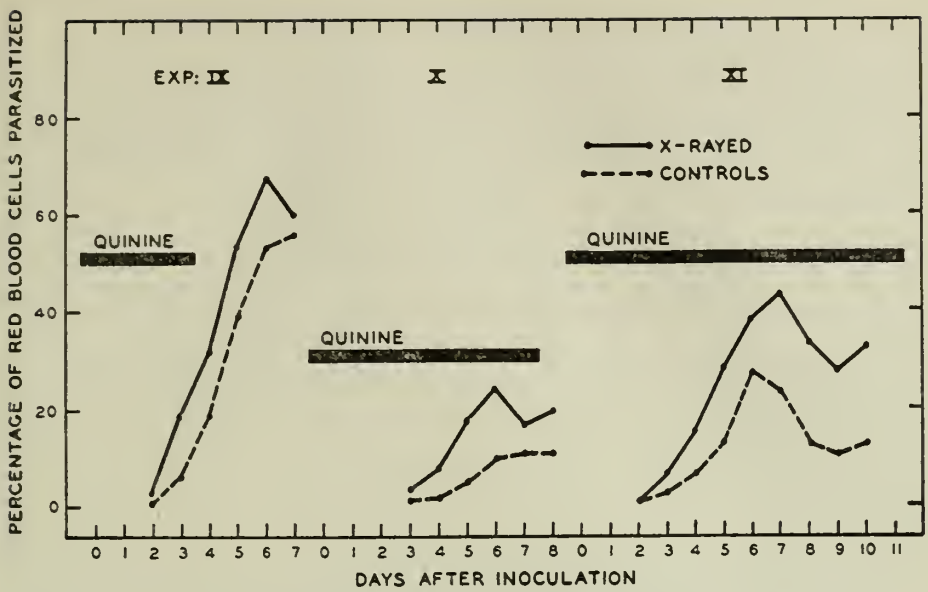

FIG. 3. Average daily parasite counts in irradiated and control chicks treated with quinine for 4, 8 and 11 days, respectively and infected with *Plasmodium gallinaceum*.

TABLE 4

Effect of the sex of the host on parasitemia in irradiated chicks infected with Plasmodium gallinaceum

EXP.	SEX	NO. OF CHICKS	MEAN PER CENT ERYTHROCYTES PARASITIZED ON DAY					
			3	4	5	6	7	8
I (A, B & C)	Female	12			20.4	44.6	73.9	75.6
	Male	14			12.3	24.9	62.3	76.6
	$\frac{D^*}{SE_D}$				2.46	3.51	N.S.	N.S.
II	Female	7	26.1	80.3	87.3			
	Male	9	23.3	68.2	85.2			
	$\frac{D}{SE_D}$		N.S.	2.77	N.S.			
III	Female	5	30.0	79.6	86.3			
	Male	5	19.8	70.5	91.5			
	$\frac{D}{SE_D}$		N.S.	N.S.	N.S.			
V	Female	12	27.3	63.6	76.8	72.0		
	Male	11	14.9	54.1	73.5	62.0		
	$\frac{D}{SE_D}$		4.05	N.S.	N.S.	2.26		

* $\frac{D}{SE_D}$ represents the observed difference divided by its Standard Error. Values greater than 2.0 are considered significant. N.S. indicates that the observed difference was not statistically significant.

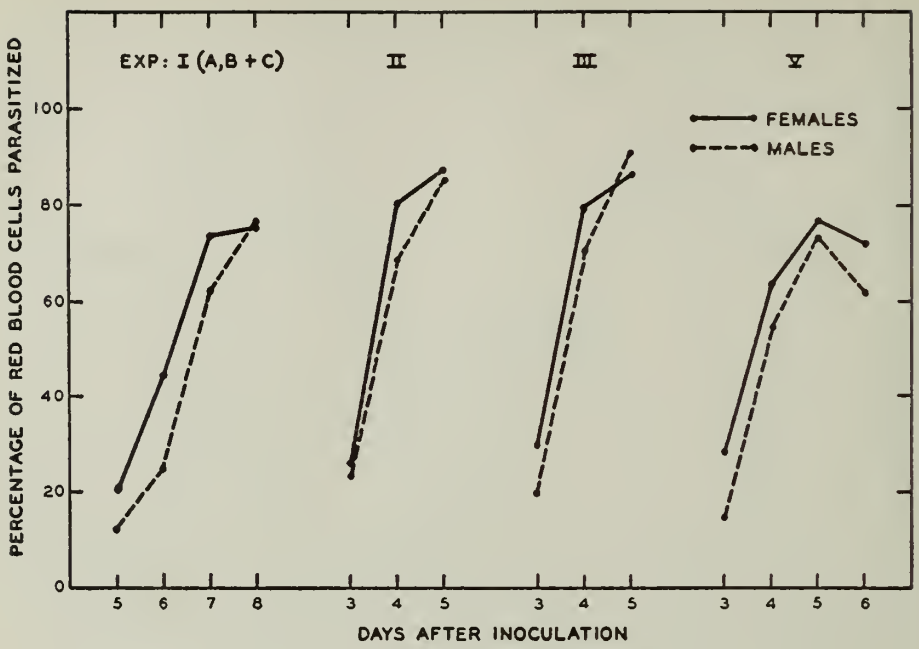

FIG. 4. Average daily parasite counts in male and female chicks exposed to X-irradiation and nfected with *Plasmodium gallinaceum.*

TABLE 5

Effect of the sex of the host on parasitemia in treated chicks infected with Plasmodium gallinaceum

EXP.	GROUP	SEX	NO. OF CHICKS	400r ON d − 1*	MEAN PER CENT ERYTHROCYTES PARASITIZED ON DAY						
					2	3	4	5	6	7	8
IX	A	Female	5	yes	2.8	24.4	39.7	66.2	81.8	79.0	
	B	Male	5	yes	1.4	10.5	21.5	37.9	50.7	41.0	
	C	Female + Male	10	no	.4	6.3	18.7	39.1	53.4	55.9	
	$\frac{D}{SE_D}$† A vs. C				2.92	3.00	N.S.	2.05	2.51	2.44	
X	A	Female	9	yes		4.3	12.5	28.3	37.0	22.8	22.7
	B	Male	6	yes		1.3	.7	.8	4.5	8.3	16.1
	C	Female + Male	19	no		.9	1.6	4.7	9.6	10.2	10.6
	$\frac{D}{SE_D}$ A vs. C					N.S.	N.S.	2.11	2.00	N.S.	N.S.
IX + X	A	Female	14	yes		11.49	22.96	41.84	53.0	38.13	
	C	Female + Male	29	no		2.74	7.49	16.99	24.2	25.43	
	$\frac{D}{SE_D}$					2.44	N.S.	2.39	2.42	N.S.	

* "r" denotes roentgens and "d" the day of inoculation.

† $\frac{D}{SE_D}$ represents the observed difference divided by its Standard Error. Values greater than 2.0 are considered significant. N.S. indicates that the observed difference was not statistically significant.

untreated controls. Experiment VIII included 35 chicks 4 days old when inoculated with 57 × 10^6 parasitized erythrocytes. Fifteen birds were exposed to 400 r on the day before inoculation and 20 were untreated controls.

As shown in Table 2 and Figure 2 the treated birds showed sign ficantly higher parasite counts than the untreated controls.

Quinine treatment of irradiated chicks infected with P. gallinaceum. Inasmuch as the foregoing experiments had shown that irradiated birds exhibited a higher parasitemia than untreated controls, it now seemed of interest to establish the combined effect of irradiation and quinine therapy.

Experiment IX included 20 young chicks. When six days old 10 of these birds were exposed to 400 r and 10 were kept as controls. On the following day, all birds were

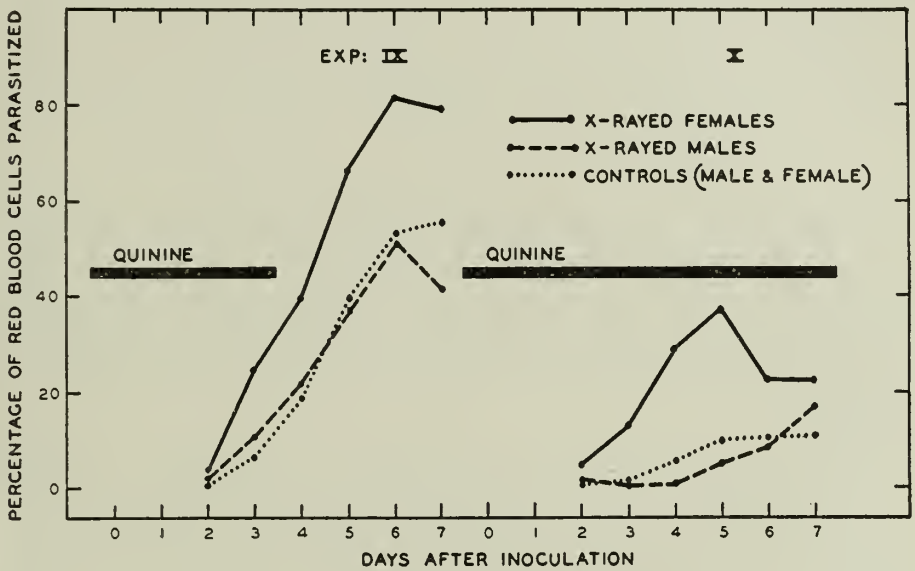

FIG. 5. Average daily parasite counts in unirradiated controls and in male and female chicks exposed to X-irradiation. All birds were infected with *Plasmodium gallinaceum* and treated with quinine for 4 or 8 days.

started on treatment with quinine sulfate (0.02 mgm./gm. body weight) given twice daily for four days, beginning four to five hours before inoculation with 16 × 10^6 erythrocytes parasitized with *P. gallinaceum.*

Experiment X included 34 chicks; 15 were irradiated and 19 were kept as unirradiated controls. The details of this experiment followed the previous experiment in every respect except that the birds received drug for a period of eight days.

Experiment XI included 36 young chicks. When seven days old 19 of these birds received 400 r and 17 served as unirradiated controls. On the same day all of the birds were started on treatment with quinine sulfate (0.02 mgm./gm. body weight) given twice daily for 11 days, beginning four to five hours before inoculation with 10^7 parasitized erythrocytes.

As shown in Table 3 and Figure 3 quinine sulfate was less effective in controlling the parasitemia in irradiated chicks than in those which received no irradiation.

Sex differences in parasite counts of irradiated chicks infected with P. gallinaceum. In an earlier study (Bennison and Coatney, 1948) it was shown that female chicks infected with *P. gallinaceum* exhibited higher parasite counts and were less effectively protected by quinine than were the males. It therefore seemed advisable to analyse the data in the present study on the basis of the sex of the birds. When this was done for Experiments I, II, III and V (Table 4 and Figure 4), it was found that the irradiated female chicks showed significantly higher parasite counts than the males that were treated similarly.

The sex difference is more pronounced in the groups of chicks of Experiments IX and X which were irradiated and received quinine. Reference to Table 5 and Figure 5 will show that irradiated males exhibited parasite counts essentially equivalent to the quinine-treated controls, but the irradiated females showed much higher counts than either the quinine-treated males or the unirradiated male and female controls. The increase in average parasite counts of irradiated quinine-treated chicks as compared with unirradiated quinine-treated controls may be due almost entirely to the increased parasitemia in the female chicks.

DISCUSSION

The results presented above clearly indicate that X-irradiation of chicks inoculated with *Plasmodium gallinaceum* trophozoites is associated with significantly higher parasite counts. That this effect is not limited to a single species of parasite, however, is indicated by the increased counts observed in X-rayed chicks inoculated with *P. lophurae.* The effect is also observed in birds given minimal effective dosages of quinine sulfate and is particularly striking in female chicks under the latter circumstances.

Taliaferro *et al.* (1945) have discussed the possible explanation of the increased severity of infections in X-rayed hosts, and conclude that the effects are probably due to a destruction of lymphocytes, which in turn "would not only deplete an important mesenchymal reserve from which macrophages are mobilized but also, possibly, one source of antibodies." We have no reason to dispute such a conclusion but, nevertheless, we find it difficult to explain the difference in the parasite counts between the male and female irradiated birds in our quinine-treated series on such a basis.

One experimental application of X-irradiation lies in the possible adaptation of parasites to a poorly susceptible host. Zinsser and Castaneda (1932) and Naiman (1944) and others have suggested such a procedure to tip the balance in favor of the parasite. In addition to the work reported here we have carried out two experiments with *P. relictum* of the pigeon and dove (Coatney, 1938) which grows very poorly in the chick. These studies were too limited for definite conclusions, but it can be said that the irradiated birds developed a greater parasitemia and the infections continued longer in them than in the untreated controls. In further studies of host susceptibility, granting the preliminary nature of the *P. relictum* studies, added success might be obtained if, in addition to the irradiation, only young female hosts were employed because of their greater susceptibility as shown in this and our previous study (Bennison and Coatney, 1948).

SUMMARY

1. Chicks exposed to 400 roentgens on or about the day of inoculation with either *Plasmodium gallinaceum* or *P. lophurae* showed significantly higher parasite counts than the unirradiated controls.

2. Parasite counts of irradiated quinine-treated chicks, inoculated with *P. gallinaceum*, averaged significantly higher than those of unirradiated quinine-treated controls.

3. Parasite counts of X-rayed female chicks averaged significantly higher than those of irradiated males, with and without quinine treatment.

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OBSERVATIONS ON *PLASMODIUM LOPHURAE* INFECTIONS IN WHITE PEKIN DUCKLINGS TRANSFUSED WITH DUCK AND CHICK ERYTHROCYTES[1]

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Rigdon and Varnadoe (1945) gave a series of transfusions of normal duck erythrocytes to eleven ducklings with *Plasmodium lophurae* infection, beginning early on the sixth day when the parasite population was at or near its peak, and observed that the parasitemia could be maintained at a high level, providing sufficient normal erythrocytes were injected, instead of declining rapidly as in non-transfused birds developing red-cell anemia from destruction of cells by the parasite. They commented that since the decrease in the number of parasitized erythrocytes following the peak of the infection had been considered by many investigators to be a manifestation of immunity, and since there was no reason why the immunity mechanism (phagocytosis) could not proceed normally in clearing the blood stream of transfused ducklings of parasitized red cells, factors other than phagocytosis might be operative in producing the decrease in parasitemia following the peak of the infection.

The same authors followed the appearance of young red cells in the blood after anemia commenced to develop, in one case from the fourth to the fifteenth day, and commented that it was interesting to observe the rapidity with which these young cells became mature. Later, however, Rostorfer and Rigdon (1947) developed certain doubts as to the continuity of the erythroblasts in the anemic bird with the normal adult red cells that succeeded them in the blood of recovered ducks. Present interest in the young red cells arises from the statements by Rigdon (1947) and Rigdon and McCain (1947) that *P. lophurae* prefers adult erythrocytes to young erythrocytes. The significance of their statement was that the large numbers of young red cells present after the peak of the parasitemia may be one of the "factors other than immunologic" producing the death of *P. lophurae* in ducks.

The present paper contains a verification of Rigdon and Varnadoe's findings regarding the postponement of the decline of the parasitemia in *P. lophurae* infection by red blood cell transfusions. In addition, it is shown that erythrocytes from immune ducks make as suitable hosts for *P. lophurae* as erythrocytes from normal ducks. Other contributions are certain observations on the effect of transfusing chicken cells into ducks with *P. lophurae* infection, and the results of a study of the parasitism of young red cells.

METHODS

Our methods were practically the same as those employed by Rigdon and Varnadoe. The criterion of intensity of parasitemia was per cent of parasitized red blood cells

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instead of number of parasites in 500 red blood cells. Other details appear in the discussions of the series.

RESULTS

Series 1. In this series there were four ducks which were injected with washed erythrocytes from normal half-grown chickens, four injected with once washed (in physiological salt solution) erythrocytes from normal ducks which were about two-thirds grown, and eight controls not transfused. Age of ducks, number of parasitized cells injected to inoculate each duck, volume of red cells injected, and other data appear in Table 1.

Two (Nos. 75 and 76) of the ducks transfused with chicken erythrocytes died on the fifth day, after the second transfusion, but ducks No. 77 and No. 78 survived the transfusions of days six and seven and were found dead on the morning of day eight. Since the cycle of *Plasmodium lophurae* is about 36 hours, according to a number of authors among whom the most recent are Taliaferro and Taliaferro (1948), it is altogether likely that on days six and seven the chick erythrocytes injected into ducks No. 77 and No. 78 were serving as hosts for *P. lophurae*, because the total erythrocyte counts were being fairly well maintained and the percentages of infected cells were moderately high, i.e., 44 per cent and 45 per cent, respectively, on day six and 27 per cent and 38 per cent, respectively, on day seven.

The plasma was separated from the red cells administered to ducks No. 79–82. Ducks No. 80 and No. 82 survived through the tenth day, but duck No. 79 died on the seventy-seventh day of causes unknown and duck No. 81 died on the twenty-fourth day with a high parasitemia. It was necessary to treat duck No. 79 with quinine six days before its death on account of a severe recrudescence of the parasitemia, but the infection had subsided in response to the treatment several days before death occurred. The controls did not fare so well, since four of the eight were found dead on the morning of the sixth day, two more were dead on the morning of the seventh day, another was dead on the fifteenth day, but duck No. 85 survived until the thirty-second day when it was killed because it exhibited lack of coordination in its movements. The parasitized cell count at this time was only 0.03 per cent.

Series 2. The pertinent data for this series of twelve ducks appear in Table 2. The ducks of Groups 1 and 2 were transfused with red blood cells from a group of ten "immune" ducks. The latter had been inoculated 40 days previously when thirteen days of age, and, in order to pull them through the acute attack, had been administered quinine when the parasitemia reached about thirty-five per cent. No quinine was administered after the tenth day to any of the four ducks.

The red cells administered to Group 1 were washed once with physiological saline, while those administered to Group 2 were resuspended in the original plasma. It will be noted that in this series transfusions were not commenced until the morning of the sixth day and were continued only until the afternoon of the ninth day. As shown in Table 2, the total counts were better maintained on the seventh day in Groups 1 and 2 than in Group 3, the non-transfused controls. The same was true also on the eighth day by which time there had been one death in each of Group 1 and Group 2, and two in Group 3, the controls. The parasitemias, in general, were

TABLE 1

Series 1: Records of total erythrocyte counts, percentages of parasitized cells, age of ducklings when inoculated, numbers of parasitized duck cells injected per 100 g. of body weight, and volumes of red cells injected. (Age, 14 days; 2.5×10^8 P. C.)

GROUP	DUCK	DAYS AFTER INOCULATION																									
		1	3		4		5		6		7		8		9		10		11		12		15		23	
		T.C.	P.C.	T.C.	P.C.	T.C.	P.C.	T.C.	P.C.	T.C.	P.C.	T.C.	P.C.	T.C.	P.C.	T.C.	P.C.	T.C.	P.C.	T.C.	P.C.	T.C.	P.C.	T.C.	P.C.	T.C.
A. Erythrocyte counts (P. C. = per cent parasitized cells; T. C. = total RBCs per mm.³ in millions)																											
1 (chick RBCs)	75	2.6	20	2.4	64	2.1	74	1.5	D																		
	76	2.1	28	2.0	62	2.0	69	1.2	D																		
	77	1.9	19	2.0	64	1.7	72	1.3	44	1.2	27	1.5	D														
	78	2.3	27	2.2	61	1.9	72	1.7	45	1.5	38	1.4	D														
2 (normal duck RBCs)	79	2.2	17	2.5	69	2.0	62	1.5	85	1.2	50	1.9	59	1.5	76	0.9	37	0.5	5	0.6	2.3	1.5	3.0	2.4	0.0	2.3
	80	2.4	19	1.9	67	2.1	72	1.8	59	0.9	31	1.5	17	1.9	54	1.9	68	1.3	D							
	81	2.3	31	2.7	46	2.3	87	1.7	56	1.6	47	2.8	69	2.7	83	2.4	49	1.8	83	0.4	44	1.7	50	1.7	60	0.7
	82	2.1	25	1.8	63	1.3	82	1.0	54	1.5	24	0.9	44	2.7	67	2.1	78	1.1	D							
3 (controls, not transfused)	83	2.1	22	2.2	71	1.9	85	1.2	D																	
	84	2.6	25	2.4	62	2.1	76	2.1	D																	
	85	2.3	26	2.2	62	2.1	76	1.5	65	0.7	56	0.6	30	0.6	5	0.6	+	0.5	0.1	0.7	0.0	0.9	0.0	1.7	+	2.1
	86	2.3	24	1.9	60	1.7	73	1.4	60	0.7	D															
	87	2.7	25	—	56	—	44	—	61	0.8	66	0.5	66	0.4	61	0.3	55	0.5	53	0.7	55	0.3	D			
	88	2.7	25	—	62	—	86	—	D																	
	89	2.7	28	—	68	—	85	—	D																	
	90	2.8	18	—	71	—	69	—	45	0.5	D															

B. Injections of loosely packed RBCs (A = A.M.; P = P.M.)

Day	5		6					7		8		9		10	11	12	13–18
Hour	10:00A	5:00P	6:30A	10:00A	3:00P	6:30P	11:00P	7:00A	4:30P	11:00A	3:00P	10:00A	4:30P	9:30A	9:30A	9:30A	9:30A
cc	1.5	2.0	4.0	3.5	3.5	2.5	4.0	3.5	3.0	3.5	3.0	3.5	3.0	5.2	4.0	3.5	2.5

TABLE 2

Series 2: Records of total erythrocyte counts, percentages of parasitized cells, age of ducklings when inoculated, numbers of parasitized duck cells injected per 100 g. of body weight, and volumes of red cells injected. (Age, 12 days; 1×10^8 P. C.)

GROUP	DUCK	DAYS AFTER INOCULATION																		
		0	2		4		5		6		7		8		9		10		21	
		T.C.	P.C.	T.C.	P.C.	T.C.	P.C.	T.C.	P.C.	T.C.	P.C.	T.C.	P.C.	T.C.	P.C.	T.C.	P.C.	T.C.	P.C.	T.C.
A. Erythrocyte counts (P.C. = per cent parasitized cells; T.C. = total RBCs per mm.³ in millions)																				
1	151	2.9	1.1	—	24	2.1	28	2.1	73	1.5	46	2.5	80	1.6	85	0.6	D			
	152	2.3	1.2	—	30	2.2	54	2.4	86	1.5	36	2.2	D							
	153	2.0	1.0	—	33	2.1	52	2.3	82	1.5	76	2.2	63	0.9	D					
	154	2.4	1.3	—	20	2.0	40	1.8	76	1.9	81	1.3	57	1.0	59	0.7	17	2.0	0.1	2.4
2	155	3.3	1.2	—	31	1.8	53	1.6	86	1.1	64	1.7	D							
	156	2.2	1.1	—	14	2.2	10	2.2	65	1.8	56	3.2	81	2.3	71	0.7				
	157	1.9	1.2	—	26	2.3	44	2.1	89	1.5	70	1.5	82	1.8	D					
	158	2.6	1.2	—	27	2.3	47	1.5	84	1.3	57	1.4	57	0.9	D					
3 (controls, not transfused)	187	2.3	1.3	—	23	2.4	39	2.2	79	2.6	69	0.8	59	0.7	D					
	188	2.6	1.0	—	28	2.3	41	2.4	74	1.7	66	1.2	D							
	189	2.2	1.0	—	22	2.8	33	2.4	82	2.9	78	0.5	D							
	190	2.4	1.0	—	34	1.8	54	1.8	82	1.1	76	0.5	41	0.8	31	0.8	33	0.7	0.6	1.9

B. Injections of loosely packed RBCs (A = A.M.; P = P.M.)

Day	6					7			8	9		
Hour	8:30A	11:00A	2:00P	8:00P	11:00P	9:00A	11:30A	5:00P	8:00A	9:00P	1:00P	3:00P
cc	2.5	1.5	1.6	1.5	2.0	2.5	2.0	2.0	2.0	4.0	2.5	2.5

also higher in the six survivors of Groups 1 and 2 than in the two survivors of Group 3, despite the higher total erythrocyte counts, indicating that more cells per volume of blood were being utilized as host-cells. By the morning of the ninth day there were but three survivors among the transfused ducks and but one among the controls, but the former had markedly higher parasitemias. Duck No. 154 survived until the fifty-eighth day, when it was killed, but experienced several mild recrudescences during this interval. Duck No. 190 died on the 163rd day of its infection of causes unknown after experiencing a post-crisis infection of the intermittent type (Becker, Brodine and Clappison, 1949).

Series 3. This series consisted of but two ducklings both of which received injections of erythrocytes from "immune" ducks as in Series 2, except that the administrations of erythrocytes were prosecuted more intensively, much as in Series 1. Since the only object of the experiment was to prolong the lives of the birds beyond those of the immune-cell recipients of Series 2, no records of total erythrocyte counts or per cent of parasitized erythrocytes were kept until the ninth day. On this day duck No. 1 had a total red cell count (T. C.) of 1,200,000 and the per cent of parasitized erythrocytes (P. C.) was 61. The T. C. for duck No. 2 was 2,000,000 and the P. C. was 81. On the morning of the tenth day the T. C. and P. C. for duck No. 1 were, respectively, 1,100,000 and 58, and for duck No. 2 they were, respectively, 1,800,000 and 74. Both ducks were found dead on the morning of the eleventh day, probably because of the time that elapsed since the last transfusion which was on the afternoon of the ninth day.

Infection of young red cells. The stained slides of ducks Nos. 79, 81, 85 and 87, which constituted the longest survivors of Series 1, were selected for a special comparative study of the incidence of *Plasmodium lophurae* in adult and young red cells. The slides were stained in Giemsa and the incidence of immature cells was based on observation of no less than 300 adult and young cells combined. The incidence of infected young cells was based on the number of parasitized cells among the number of young cells in a total of 300 erythrocytes. The results of the counts for ducks No. 81, one of those transfused with normal duck erythrocytes, and No. 85, not transfused, appear in Table 3.

The transfused duck, No. 81, proved to be an especially favorable host for the study because it seemed to be kept alive only by virtue of the transfusions, since it developed no protective immunity as did duck No. 79. It is to be noted that the incidence of parasitized cells (P. C.) both mature and immature, from the fifth to the twenty-third days, inclusive, varied from 44–87 per cent. The incidence of parasitism in the immature cells alone during the same period varied from 0–72 per cent. It is interesting that on days 22 and 23, when 57 and 64 per cent, respectively, of the red cells were young, 43.8 and 64.2 per cent, respectively, of the immature cells were parasitized. On day 23 there were actually more parasitized young red cells in the blood than parasitized adult erythrocytes!

The non-transfused duck, No. 85, did not exhibit so striking a parasitism of its red cells, but on the seventh day, at the onset of the period of acute anemia, when the incidence of immature red cells reached a peak of 54.5 per cent, between a fifth and a sixth of the immature red cells contained *P. lophurae*. The record for duck

No. 79, which was able to develop an active protective immunity, and the record of duck No. 87, not transfused, were more like that of duck No. 85 insofar as the incidence of parasitism in young red cells is concerned. It should be stated that both basophil erythroblasts and polychromatophil erythroblasts may be found parasitized. The interested reader will find excellent colored plates of the cells of the blood of ducks in a paper by Hewitt (1942).

TABLE 3*

Immature cell counts for 2 ducks of Series 1

T.C. = total erythrocyte count, 10,000; P.C. = parasitized cells; I.E. = immature erythrocytes; I.I.E. = infected immature erythrocytes; A = A.M.; P = P.M.; + = positive, but < 0.5 percent.

DAY OF INFECTION	DUCK NUMBER 81 (TRANSFUSED)				DUCK NUMBER 85 (NOT TRANSFUSED)			
	T.C.	P.C.	I.E.	I.I.E.	T.C.	P.C.	I.E.	I.I.E.
		per cent	*per cent*	*per cent*		*per cent*	*per cent*	*per cent*
1	225	—	—	—	230	—	—	—
3	272	31	0	0	220	25	5.0	0
4	231	46	0	0	206	62	8.0	8.3
5	169	87	12.3	19.0	154	76	7.7	13.0
6	162	85	12.3	67.6	73	65	26.0	15.3
7 A	279	47	5.7	15.7	55	56	27.7	16.8
7 P	224	58	6.0	41.1	59	52	54.5	18.9
8	274	59	5.0	60.0	56	30	34.0	3.9
9	244	83	1.3	25.0	58	5	27.0	0
10	182	49	2.3	72.0	52	+	21.0	0
11	42	83	13.3	20.0	66	+	34.7	0
12	173	44	6.0	0	89	0	28.3	0
13	65	45	17.0	0	156	0	24.0	0
14	131	58	22.0	45.5	180	0	9.7	0
15	165	50	29.7	29.2	166	0	5.0	0
16	84	68	29.0	16.0	172	1	3.0	0
17	137	84	7.0	43.0	195	+	1.0	0
18	81	84	15.5	22.0	238	0	1.0	0
19	125	77	29.5	64.5	197	+	3.0	0
21	85	68	35.6	11.3	199	0	1.0	0
22	121	65	57.0	43.8	250	+	—	—
23	70	60	64.3	64.2	205	+	3.7	0

* Apparent discrepancies between certain items in this table and Table 1 consist of observations made at different times of the day.

DISCUSSION

The amazing work of Rigdon and Varnadoe (1945) referred to in the first paragraph has been confirmed. In addition, it has been shown that red blood cells from "immune" ducks, that is, in the post-crisis of *Plasmodium lophurae* infection, probably serve as well as cells from normal ducks in maintaining the parasitemia when transfusions are commenced at about the time of the primary attack when the parasitemia is near the peak. The importance of this demonstration may not be too apparent until it is judged against the background of the conclusions reached by a number of in-

vestigators, among them the senior author, as to the nature of immunity in coccidiosis, another sporozoan infection. During many coccidian infections the host develops an active immunity to the specific coccidium concerned, but in such cases it has not been possible to demonstrate humoral antibodies. The cells of the tissues which in normal animals are suitable host-cells for the development of the coccidium become free of parasites during the development of actively acquired immunity and, in addition, either resist further invasions of sporozoites or prove unsuitable hosts for them if they succeed in entering. (The subject has been treated comprehensively by Reyer, 1941.) In such cases it is assumed that a local cellular immunity develops. Evidently immunity in *lophurae* malaria is not, even in part, of the cellular type, for the cells from immune (or premunized) ducks are, apparently, as susceptible to the invasion of the parasite as cells from ducks which have had no experience with the parasite.

It is of some interest that chick erythrocytes can serve, to some extent at least, as host cells for *P. lophurae* in the duck. The transfusions are dangerous to the ducklings, however, and they develop fatal anaphylactic reactions.

It has been established that immature red blood cells of the duck (basophil erythroblasts and polychromatophil erythroblasts) may serve as host-cells for *Plasmodium lophurae* to a greater extent than several previous authors have supposed. When one considers the promptness with which these young cells appear in the blood when anemia begins and the rapidity with which they either become mature or are replaced in the blood with normal adult erythrocytes (see second paragraph) in connection with the observations on the incidence of parasitism of these cells, e.g., as reported in Table 3 for duck No. 81, then it would seem that the burden of proof is on those who declare that adult erythrocytes are more suitable hosts for *P. lophurae* than the younger red blood cells.

It is of some interest here that McGhee (1949) obtained infections of *P. lophurae* in the erythrocytes of Swiss mice introduced intravenously into chick embryos previously inoculated with the malarial organism.

CONCLUSIONS

1. It is confirmed that in *Plasmodium lophurae* infection the parasitemia in ducklings at or near the crisis can be maintained by transfusing normal duck erythrocytes.

2. Erythrocytes of "immune" (i.e., post-crisis) ducks, when transfused into ducklings at or near the crisis, prove as suitable host-cells for *P. lophurae* as erythrocytes from normal ducks.

3. Actively acquired resistance in *lophurae*-malaria, therefore, is not resident wholly or in part within the red blood cell, but is concerned with other tissues.

4. Transfused chick erythrocytes may serve, to some extent at least, as host-cells for *Plasmodium lophurae* in the blood stream of the duck.

5. Immature red blood cells of the duck are more suitable hosts for *Plasmodium lophurae* than has been previously appreciated.

6. The decline in incidence of parasitized red blood cells following the peak of the

parasitemia cannot, therefore, be attributed in any considerable part to the aversion of the parasite for young red blood cells.

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EFFECT OF HUMAN BLOOD ON *PLASMODIUM KNOWLESI* INFECTION IN MONKEYS[1]

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It has been shown that the parasitemia in ducks infected with *Plasmodium lophurae* may be kept at a high level by the intravenous injection of duck blood (Rigdon and Varnadoe 1945). This was thought to result from the addition of homologous red cells to the circulation when the birds were anemic from the destruction of their cells by the plasmodia. In contrast to this effect on the parasitemia by homologous blood, it has been observed that the parasitemia produced by *Plasmodium knowlesi* in the monkey may decrease 36 to 48 hours following the injection of heterologous blood (Rigdon 1948).

The present experiments were made to study the mechanism of the effects of human blood on *P. knowlesi* infection in monkeys. Human plasma, human hemoglobin, and bovine and horse serum also were used.

METHODS AND MATERIALS

Twenty-nine *Macaca mulatta* monkeys were used. *P. knowlesi* was obtained from donor monkeys and inoculated either intravenously or subcutaneously. The degree of parasitemia was determined by counting the number of parasitized cells per 500 erythrocytes after staining the smears with a combination of Giemsa's and Wright's stains. Red blood cell counts were made by standard techniques. The frequency in which these counts were made and the degree of parasitemia are shown in the different experiments.

Blood was obtained from the Blood Bank[2] and represents that collected from syphilitic donors or "out-dated" specimens. No attention was given to the blood group of either the donor or recipitant. Blood was injected both intravenously and intraperitoneally. The largest amount given at one time was 120 cc (60 cc I.V. and 60 cc I.P.) to a monkey that weighed 3.5 Kg. The maximum given intravenously within a period of 24 hours was 150 cc to a monkey that weighed 3.7 Kg. The time used to give the transfusions was 10–15 minutes. The number of blood transfusions, the quantity, and the number of days during which they were carried out are shown in the specific experiments.

The hemoglobin was obtained by rapidly freezing and thawing whole human blood twice in dry ice and alcohol. The preparation was then centrifuged and the supernatant used for injection. The hemoglobin was given intravenously. The

[1] Aided by a Grant from the John and Mary R. Markle Foundation.

[2] We wish to thank Dr. W. C. Levin for this blood.

maximum amount given at one time was 50.0 cc. The quantity and the frequency of the injections are given in the experiments.

The human plasma was dehydrated and subsequently dissolved in distilled water. The maximum amount given to a monkey was 235 cc over a period of three days. The largest amount injected at one time was 110 cc (70 cc I.P. and 40 cc I.V.). Some animals were immunized with human plasma before they were injected with *P. knowlesi*, while others were treated with plasma during the period of malarial infection.

Horse and bovine serum were obtained from a commercial laboratory.[3] The sera were given intraperitoneally to immunize monkeys before they were infected with *P. knowlesi*. The details of these experiments are given later.

EXPERIMENTAL RESULTS

Observations on Development of Sensitivity in Monkeys to Human Blood, Plasma and Foreign Sera

Thirteen monkeys were transfused with human blood. The amount, time of injection and route is shown in Table 1. A majority of these monkeys were transfused on three or four consecutive days; however, two monkeys, 4 and 5, were transfused at intervals of one or more years. In none of these monkeys did we observe any reaction to the human blood.

Four monkeys, 11, 12, 13 and 14, were given human plasma as follows: 50.0 cc intraperitoneally to two monekys on four consecutive days. A skin test was made approximately four months later and there was no reaction. One monkey, 14, was given 5.0 cc of plasma intraperitoneally on the following experimental days: 0, 1, 7, 14 and 21. There was no reaction. A second monkey, 11, was given 5.0 cc of human plasma on the following days: 0, 1, 7, 12, 21, 92 and 93 with no reaction occurring.

Two monkeys, 9 and 10, were given 5.0 cc of horse serum intraperitoneally on the following experimental days: 0, 7, 15, 22, 28, and on the 35th day 0.1 cc horse serum was injected intradermally. No reaction occurred. Additional intraperitoneal injections of horse serum were given to monkey 9 on the 77th, 84th and 90th experimental day without any reaction. One of these two monkeys, 10, was given 5.0 cc of horse serum intraperitoneally on the 137th day of the experiment without any reaction.

Two monkeys, 7 and 8, were tested intradermally with 0.1 cc of both horse and bovine serum. There were negative reactions. These two animals then were given 5.0 cc of bovine serum intraperitoneally on the following experimental days: 0, 7, 15 22 and 28. On the 35th day 0.1 cc of bovine serum was injected intradermally without producing any reaction. Additional bovine serum was given on the 77th, 84th and 90th day without any reaction. Skin tests were made on monkey 7 on the 150th experimental day using 0.1 cc each of horse and bovine serum and human plasma. Two hours later edema (3 x 5 cm) was present where the horse serum was injected. Twenty-four hours later the area of edema was 4 x 7 cm. After the same interval the

[3] Supplied by the Lederle Laboratories, Pearl River, N. Y.

area injected with bovine serum was edematous (2 x 2.5 cm) and 24 hours later it was 5 x 2 cm. Human plasma was injected intradermally in this monkey, 7, but no reaction occurred. The second monkey in this group, 8, was skin tested on the 154th

TABLE 1

MONKEY NO.	HUMAN BLOOD IN CC GIVEN TO MONKEYS												
	Experimental Day												
	0	1	2	3	4	6	8	11	607	608	609	610	787
4	50P*		60P						50V	15V 30V	30V	40V	
5	70P	50P	50P						10V 30P				10V
12	50P		30P			30P	50P						
21	55P	40P 40P	50P			60P							
23	60V 60P	50V 50P 50V	20V 40P 70V										
24	40V	60V 60P	60V										
25	60V	70V	80V 60V	60P 10V									
27	80V	80V	80P 30V	80P									
29	50V	60V	70V										
31	24V 32V	30V			34V			10V					
86	50P	45P	40P	40P									
125	60P	50P	40V										
152	60P	60P	60P										

* P—Intraperitoneal; V—Intravenous injections.

experimental day but no reaction occurred to either horse and bovine serum or human plasma.

During these experimental studies changes did occur in one monkey that suggested a sensitivity type of reaction. Monkey 9, previously discussed, had been given horse

serum intraperitoneally over a period of three months with no reaction. On the 56th day following the last injection of horse serum this monkey was inoculated subcutaneously with 1 cc of parasitized blood obtained from monkey 10. This latter monkey had received a series of injections of horse serum and the last injection was eight days previously. Forty-eight hours after the subcutaneous injection of the blood from monkey 10, the site of inoculation in monkey 9 was edematous. This edema progressively increased to involve the external genitalia, eyes, face and neck. Monkey 9 died seven days following the subcutaneous injection of 1.0 cc of blood from monkey 10. At autopsy there was extensive subcutaneous edema, but no fluid was present in the serous cavities. Death was considered to have resulted from a sensitivity reaction such as occurs in the Arthus' phenomenon. No parasites were demonstrated in the peripheral blood.

Effect of Intravenous Injected Human Hemoglobin on Monkeys

From the preceding observations on sensitivity it would seem that normal monkeys are unlikely to be sensitive to human blood and, too, they are unlikely to develop any sensitivity following multiple injections of human blood.

Seven monkeys, 31, 32, 36, 37, 42, 43 and 46, were given one or more intravenous injections of human hemoglobin. Two of these, 42 and 43, were given 45.0 and 50.0 cc each in one injection. Monkeys 36 and 37 were given 0.5 to 5.0 cc of hemoglobin intravenously at intervals of five to ten days over a period of approximately two months. No reactions were observed to develop in any of these four monkeys. Monkey 46 was given 50.0 cc of hemoglobin intravenously, and one hour later petechaie were present in the skin and the face was edematous. These lesions subsided within 24 hours. Four days following the first injection of hemoglobin, 0.5 cc of hemoglobin was injected intradermally. No reaction occurred. Forty days later 10.0 cc of hemoglobin was given intravenously. Only a questionable amount of edema and hyperemia developed about the face. Monkey 46 is the only one that developed a sensitivity type of reaction following the injection of human hemoglobin. Furthermore, it is interesting to note that the second injection of hemoglobin, forty days after the first, produced a much less severe reaction than the first.

Monkeys 31 and 32 were given the amounts of human hemoglobin and human blood as shown in table on page 302.

Neither of these two monkeys showed any reaction that suggested sensitivity. Both animals, however, died unexpectedly during the night. No significant gross lesions were found at autopsy. Hemoglobin casts were present in some of the renal tubules. The exact cause of death was not determined.

Effect of horse and bovine serum on the parasitemia produced by P. knowlesi *in the monkey*

One monkey, 10, was given horse serum and two monkeys, 7 and 8, were given bovine serum before inoculation with *P. knowlesi*. These three monkeys were given either eight or nine intraperitoneal injections of 5.0 cc each of the serum over a

period of three months. Monkey 10 was inoculated with blood containing *P. knowlesi* eight days following the last intraperitoneal injection of horse serum; monkeys 7 and 8 were inoculated with malaria 70 days after the last injection of bovine serum. The parasitemia in these three monkeys was similar to that occurring in normal monkeys similarly inoculated.

EXP. DAY	MONKEY 31		MONKEY 32
	Hemoglobin	Blood	Hemoglobin
	cc	*cc*	*cc*
0	6.0 8.0	24 32	5.0
1	10.0	30	4.5 5.0
2			5.0 5.5
3			5.5 10.0
4	6.0	34	Dead
11		10	
12	5.0 5.0		
13	6.0 5.5		
14	5.2 10.0		
15	Dead		

Effect of human plasma on the parasitemia produced by P. knowlesi *in the monkey*

Three monkeys, 11, 12 and 13, were given human plasma before they were inoculated with *P. knowlesi.* Monkey 11 was given 5.0 cc plasma intraperitoneally on the following experimental days: 0, 1, 7, 14, 21, 93 and 94. Four days after the last injection of human plasma he was inoculated intravenously with blood containing *P. knowlesi.* Monkeys 12 and 13 were given 50.0 cc of human plasma intraperitoneally on four consecutive days. Monkey 12 was inoculated with malarial infected blood 95 days after the last injection of human plasma, and Monkey 13 was inoculated 100 days following the last injection of the human plasma. Monkeys 11 and 12 both developed the infection and nothing abnormal was observed relative to the course of the parasitemia. Monkey 13 failed to develop the infection and was reinoculated subcutaneously 15 days later. Nothing abnormal was observed in the course of the

parasitemia in this monkey. Since normal monkeys sometimes fail to develop the infection within two weeks after subcutaneous inoculation, we do not attach too much significance to this failure.

Two monkeys, 2 and 15, were given human plasma during the time of their parasitemia. The protocol on these two animals follows:

MONKEY 2			MONKEY 15		
Exp. Day	Human Plasma in cc	Parasitized cells per 500 RBC	Exp. Day	Human Plasma in cc	Parasitized cells per 500 RBC
0	Inoculated I.V.		0	Inoculated I.V.	
2	15 I.V.	3	3		30
3	25 I.V.	2	4	70 I.P.	28
4	20 I.P.	4		25 I.P.	118
5	20 I.P.	41		50 I.P.	
6	30 I.P.	43	5	30 I.V.	
	25 I.P.	48		20 I.P.	268
7	25 I.P.	219	6	40 I.V.	404
7	Experiment discontinued		6	Died	

From these experiments there is no indication that human plasma given either before or during the period of parasitemia influences the course of *P. knowlesi* infection in the *M. mulatta* monkey.

Effect of human blood on the erythrocyte count in a normal monkey

In the preceding experiments it has been shown that monkeys can be given large amounts of human blood without a reaction. The erythrocyte count in the peripheral blood of monkey 41 transfused with human blood is shown in Figure 1. It is evident that the number of red cells in the peripheral blood increased in this monkey following the intravenous and intraperitoneal injection of human blood.

Effect of human blood on the parasitemia produced by P. knowlesi *in the monkey*

Monkeys infected with *P. knowlesi* were given human blood both before and during the period of parasitemia. In a previous paper (2) it was reported that two monkeys, 5 and 86, with *P. knowlesi* infection were given only human blood. Monkey 86 died on the 79th day of the disease with extensive tuberculosis. Parasites were present in the peripheral blood at the time of death. The second monkey, 5, survived the infection and was re-inoculated 18 months after parasites were last demonstrated in the peripheral blood. A mild infection developed following the second inoculation and subsided within a month.

Monkey 29 was given a total of 180.0 cc of human blood in three transfusions. The first transfusion was given six days after the subcutaneous inoculation of 0.5 cc of malarial infected blood. During the period that the human blood was being given no parasites were demonstrated in the peripheral blood. A graph of this monkey is shown in Figure 2. There is nothing to indicate that the parasitemia is affected by the human blood when it is given preceding the appearance of parasites in the peripheral blood.

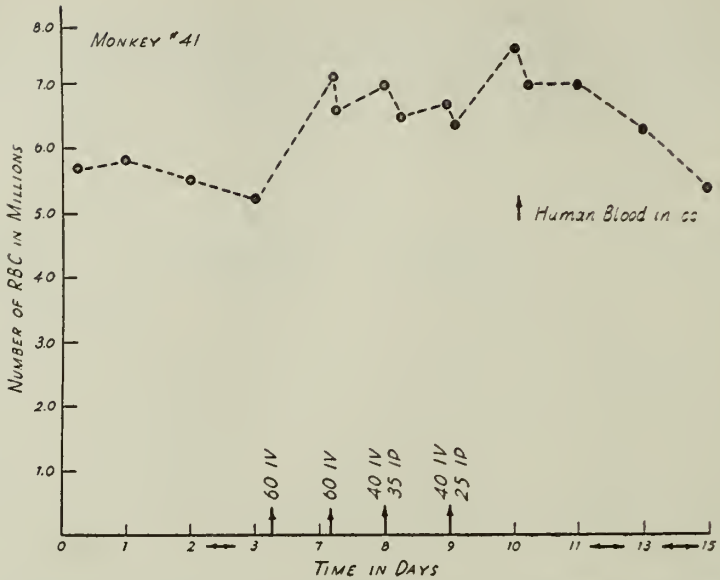

Fig. 1. *Monkey 41.* There is an increase in the number of circulating red blood cells following the intravenous and intraperitoneal injection of human blood. The erythrocyte count had returned to normal 5 days after the last transfusion.

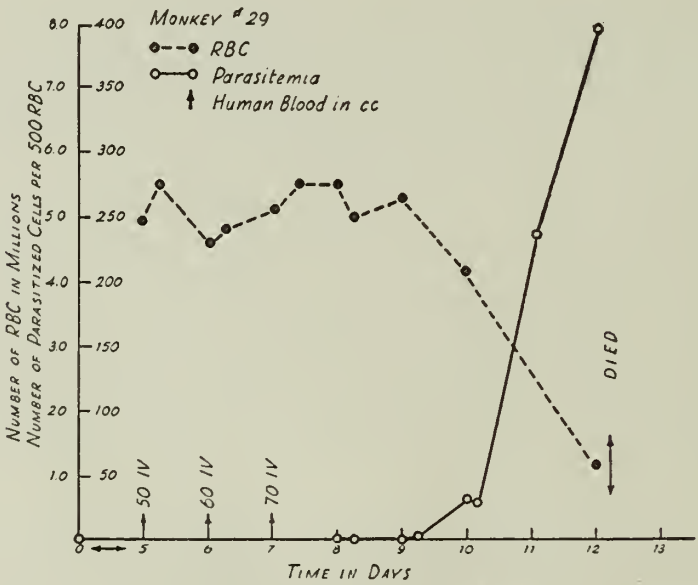

Fig. 2. *Monkey 29.* When human blood is given before the appearance of parasites in the peripheral blood the course of the parasitemia and the development of the anemia resembles that in monkey without any treatment.

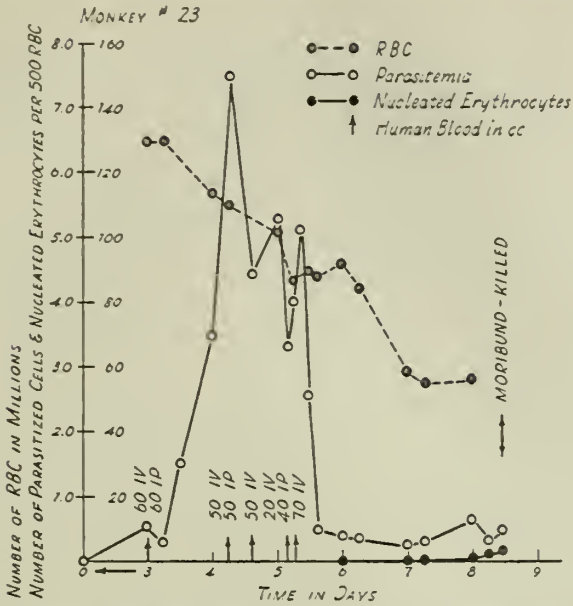

FIG. 3. *Monkey 23.* Human blood was given I. V. and I. P. at intervals during a period of approximately 60 hours. A marked decrease occurs in the degree of parasitemia following these transfusions. There are only a few parasites present in the peripheral blood during the 72 hours preceding the time of death. We have never observed such a spontaneous decrease in the degree of parasitemia in untreated *P. knowlesi* infected monkeys.

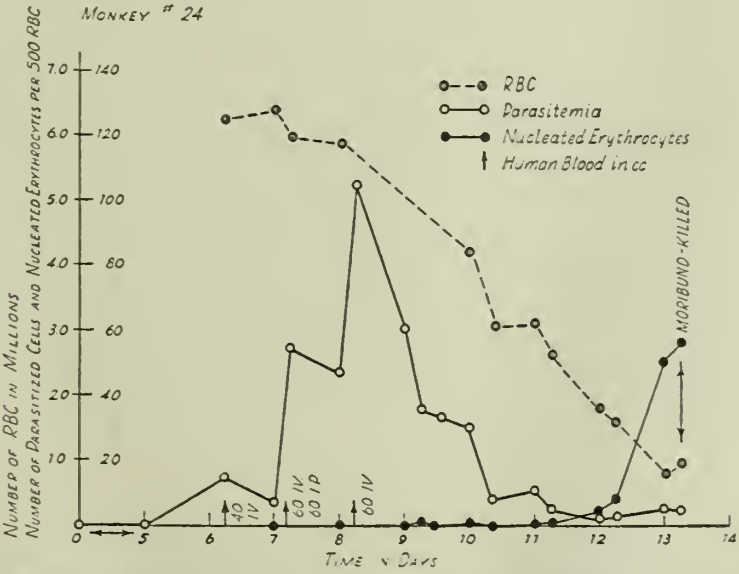

FIG. 4. *Monkey 24.* A marked decrease occurs in the number of parasitized red cells in this monkey following the injection of human blood. The number of young erythrocytes in the blood increased during the last 3 days of this monkey's life.

Three monkeys, 23, 24 and 25, were given human blood both intravenously and intraperitoneally during the time that parasites were demonstrable in the peripheral blood. The results of these three experiments are shown in Figures 3, 4 and 5. The parasitemia decreased in each of these three monkeys a short time following the first injection of blood. There was a subsequent marked decrease in the degree of parasitemia in each monkey. At the time of death the parasitemia apparently was too low to be a significant factor in the cause of death. There was a marked drop in the erythrocyte count in each of these three monkeys during the time they were receiving human blood. The anemia was severe in monkeys 24 and 25 at the time of death. There was an increase in the number of nucleated red cells in the peripheral

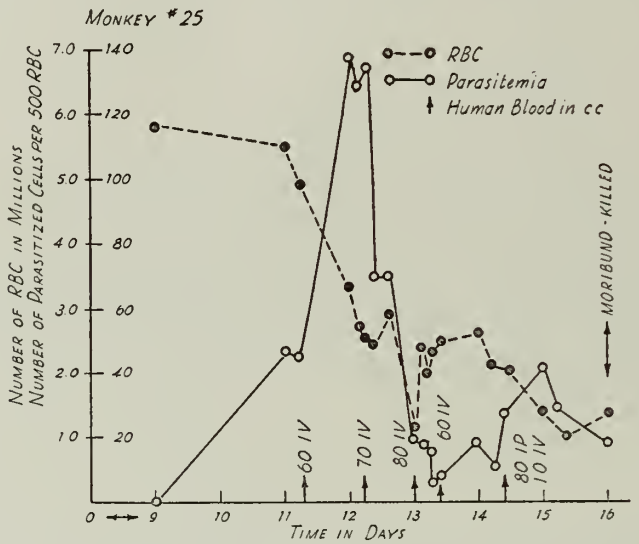

Fig. 5. *Monkey 25.* There is marked decrease in the number of parasitized cells following the injection of human blood. This decrease in parasitemia is similar to that observed in Monkeys 23 and 24.

blood of each of the three monkeys preceding the time of death. The animals were moribund at the time they were sacrificed.

The pathologic changes as observed at autopsy were typical of those occurring in *M. mulatta* monkeys dying from *P. knowlesi* infections. A small amount of blood was present in the abdominal cavity of each monkey. Hematologic studies on the femur bone marrow showed a marked increase in the percentage of young cells of the erythrocytic series.

DISCUSSION

In these experiments 25 monkeys were given one or more injections of either whole human blood, human plasma or human hemoglobin which also contained plasma. Only one monkey, 46, in this group showed any evidence of sensitivity. This reaction was characterized by a rash and edema of the face which soon subsided. It would seem, therefore, that *M. mulatta* monkeys can be given human blood with the likelihood of a sensitivity reaction occurring only infrequently.

The experiments in which human blood was given to monkeys infected with *P. knowlesi* showed a decrease in the degree of parasitemia. This decrease was marked and it persisted during the 24 to 72 hours preceding the time of death. Such a decrease in the parasitemia produced by *P. knowlesi* in untreated *M. mulatta* monkeys has not been observed by us. A decrease in the parasitemia produced by *P. knowlesi*, however, is frequently observed a short time before death. The decrease that follows the injection of human blood should not be confused, however, with that occurring immediately preceding death. A fluctuation in the number of parasitized cells in the peripheral blood in known to occur; however, the variations that occurred in this group of monkeys given human blood was much greater than the normal variations observed in untreated monkeys.

The monkeys with malaria and given human blood developed a progressive anemia. The anemia at the time of death was marked. It has been suggested that the decrease in the number of parasites in the peripheral blood of the monkeys transfused with human blood could be related to the anemia. This would seem very unlikely since the degree of parasitemia is determined by counting the number of parasitized red cells per 500 erythrocytes. The total number of parasites may decrease with a decrease in the number of red cells. However, the number of parasitized cells per 500 erythrocytes would not be affected by a decrease in the number of red cells.

Human blood given to monkeys before the appearance of parasites within the peripheral blood does not affect the subsequent course of the infection. The parasitemia in monkeys immunized with human plasma before they are infected with *P. knowlesi* does not vary from that observed in normal monkeys. Human hemoglobin likewise does not affect the parasitemia. It would seem, therefore, that whatever occurs in monkeys infected with *P. knowlesi* must be related either directly or indirectly with the human erythrocyte. It may be that this decrease in the degree of parasitemia following the transfusion of human blood is the result of an indirect action. The transfused human erythrocytes may prevent death of the monkey by supplying a medium for the transport of oxygen and carbon dioxide following the destruction of the monkey's erythrocytes by the plasmodia. In this abnormal environment the plasmodia may be unable to propagate. It has been shown elsewhere that *P. lophurae in vitro* may be severely injured by carbon dioxide and nitrogen (Rigdon and McCain 1947). The retention of metabolic products in monkeys with a severe malarial infection may be injurious to the plasmodia. It has been suggested that such a process may account for the diminution in the degree of parasitemia so often observed in monkeys a few hours before death (Rigdon and McCain 1947). It would appear to us that this decrease in the number of parasitized cells that follows the transfusion of human blood could not be accounted for only by an increase in the degree of phagocytosis. A decrease in the number of parasitized cells did not occur either in monkeys given human plasma during the period of parasitemia or in those animals immunized with human plasma preceding the time of the occurrence of parasites in the peripheral blood. Furthermore, a decrease did not occur when the blood was given before the parasites were present in the peripheral blood. Hemoglobin was not given to monkeys with malaria since the two animals given hemoglobin daily for three and four days died less than 24 hours after the last injection. The exact cause of their death was not determined. In similar experiments, Baker and

Dodds (1925) injected homologous hemoglobin into rabbits and at the end of the experiments the animals were moribund and had a marked elevation in their blood urea. The cause of death in these rabbits was thought to be the result of blockage of the renal tubules.

In a study of the mechanism of the effect of human blood on the parasitemia produced by *P. knowlesi* in the *M. mulatta* monkey it might be profitable to study the effect of heterologous blood on other hosts with malaria.

SUMMARY

Human blood when given intravenously and intraperitoneally to monkeys infected with *P. knowlesi* produces a marked diminution in the number of parasitized red cells within the circulating blood. Human plasma and hemoglobin does not effect the degree of parasitemia. The mechanism of this effect on the parasitemia is not known.

M. mulatta monkeys rarely show any evidence of sensitivity to human blood,—only one in a group of 25. Furthermore, these monkeys did not develop sensitivity to human blood after multiple injections of either blood or plasma.

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WHO MALARIA COMMITTEE REPORTS ON THIRD SESSION

The Third Session of the Expert Committee on Malaria of WHO was held in Geneva, August 10 to 17, 1949. The members present were Col. M. K. Afridi, Pakistan, Maj. Gen. Sir Gordon Covell, UK (Vice Chairman), Dr. Arnoldo Gabaldon, Venezuela, Dr. Paul F. Russell, USA (Chairman), Lt. Col. Jaswant Singh, India, Prof. N. H. Swellengrevel, Netherlands, Médecin General M. A. Vaucel, France, Dr. D. B. Wilson, Tanganyika and Dr. E. J. Pampana, (Secretary). In addition were the following co-opted members: Dr. G. R. Coatney, USA, Prof. G. MacDonald, UK, Prof. A. Missiroli, Italy. The conclusion and recommendation of this Third Session are as follows (in part):

MALARIA AND UNDERDEVELOPED AREAS

The Committee stresses again the fact that in so many tropical and sub-tropical countries malaria constitutes the greatest obstacle to development, and recommends that in any WHO programme of technical assistance to malarious underdeveloped areas, malaria control be given the highest priority.

THERAPEUTICS OF MALARIA

The Committee wishes to emphasize once again that in the communal control of malaria, interruption of transmission at the mosquito level is the method of choice. Until such effective control is accomplished there will, however, be need for providing facilities for the treatment of a large number of malaria cases. The Committee therefore urges that health officers should have up-to-date knowledge of antimalarial drugs so as to give the people the benefit of the latest advances.

In view of the continuous accretion of fresh evidence relating to antimalarial drugs, the Committee is still reluctant to give firm recommendations for the use and dosage of the new compounds but has summarized the available information which may serve as a useful guide.

The Committee considers that the World Health Organization should stimulate and co-ordinate researches on malaria therapeutic and suppressive treatment through the existing institutions rather than undertake trials on its own initiative. Such inquiries should, so far as possible, be directed along uniform lines so that the results obtained from different countries would be comparable. For this purpose certain basic principles have been listed.

The Committee recommends that the following antimalarial drugs be included in the International Pharmacopoeia:

(a) Quinine sulphate
(b) Quinine hydrochloride
(c) Mepacrine hydrochloride
(d) Mepacrine methane sulphonate
atebrine musonate
(e) Chloroquine diphosphate
(f) Proguanil hydrochloride
(g) Pamaquin naphthoate
(h) Pentaquine phosphate
(i) Isopentaquine mono-oxalate

INSECTICIDES

The Committee notes with satisfaction the setting up by WHO of the Expert Committee on Insecticides to specify international standards for insecticides and their formulations, to stimulate the development of standard spraying equipment on a regional basis and to deal with all other questions relating to the proper use of insecticides.

In order further to stimulate the use and production of insecticides and to render them available to all malarious countries, the Committee recommends:

That WHO continue to take steps to facilitate, through the existing international economic machinery, the obtaining of requests for anti-malaria supplies by governments.

That WHO explore the possibility, in its co-operative programme with UNICEF, of aiding the production of basic forms of insecticides and/or their formulations in those non-manufacturing countries in which the necessary pre-conditions exist for the economical development of such production.

The Committee suggest that WHO, with the advice of the Expert Committee on Insecticides, consider steps for circulating to governments the specifications of important insecticides and or their formulations to be used when ordering supplies.

ORGANIZATION AND PLANNING

The Committee reaffirms its opinion that the most effective safeguard against the introduction of anophelines by ships or aircraft is the rigid enforcement of anti-mosquito sanitation in and around sea and airports.

The Committee further considers that measures of disinsectization at present applicable to aircraft should be extended to sea and land traffic from places which are not free from possible malaria vectors, but lays stress on the necessity for instituting further investigations before regulations are formulated for the treatment of ships of heavy tonnage.

The Committee recommends that the relative merits and disadvantages of malaria control by residual spraying or by anopheline species eradication should be seriously considered by any country before undertaking an extensive antimalarial programme.

The Committee recommends that larval control should be abandoned in rural areas where residual insecticides are found to be effective and are extensively applied.

The Committee recommends that the calling of the malaria Conference in tropical Africa, sanctioned by the Second World Health Assembly, be given high priority.

MALARIA ACTIVITIES AND PERSPECTIVES OF WHO

The Committee, referring to the long-term objective of WHO's programme in the field of malaria (*Off. Rec. World Hlth. Org. 18*, 60), recommends that the approach to the ultimate aim should be cautiously planned, that priority should be given to economically underdeveloped malarious countries, and that in the first years of the programme particular attention should be focussed on:

Assisting governments in establishing adequate malaria control organizations, so that in the near future all malarious countries will have provision for such service, and will have at least one project for malaria control in operation.

Developing the collaboration with FAO for assisting governments in controlling malaria in food production areas where malaria interferes with agricultural and economic development.

That WHO carefully consider the possibility of itelf providing for the specialized training of selected candidates for specific positions on field projects when no suitably trained personnel is otherwise available.

That WHO should not hesitate to carry out pilot trials of malaria control by demonstration teams in areas where no pevious residual spraying has been done or where previous work leaves the matter of control by this method open to reasonable doubt or even where the identity of the malaria vector has not been proved.

That, while the demonstration teams must remain directly responsible to WHO, they should be instructed to fit themselves into the framework of the respective departments of health or of the malaria services, if such exist.

THE PERFORMANCE OF LIQUID SPRAY NOZZLES FOR AIRCRAFT INSECTICIDE APPLICATION

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An important practical problem exists in the deposition of liquid particles from aircraft for insecticidal or herbicidal purposes. There has been considerable difference in opinion as to the most practical droplet size required for the control of *Anopheles* larvae through aircraft dispersal of DDT solutions. This controversy is very much the same as that experienced some years ago with airplane dusting of Paris green. At that time, correlations were made between dosage recovery, particle size, and toxicity to *Anopheles* larvae. Metcalf and Hess (1944) reported that agricultural formulations of Paris green consisted of such fine particles that 75 per cent of the toxic material drifted away from the treatment swath. By increasing the 20 to 50 micron fraction from 48 to 84 per cent, recovery was increased by 60 per cent and excellent kills were obtained in field tests, although "peaking" in the center of the swath was greatly increased. It becomes apparent that airplane equipment for the distribution of liquid or solid particles must undergo adjustments to meet specific treatment problems. The final particle size composition adopted will be a compromise between optima for maximum recovery, swath width, penetration of vegetation, and toxicity to anopheline larvae without damage to fish food organisms.

Within the experience of the Tennessee Valley Authority, several years of field operations and considerable experimental data have been amassed on the performance of aircraft thermal aerosols. These exhaust generators, described by Kruse and Metcalf (1946) were designed to produce mass median diameter (MMD) ranges of 35 to 50 microns. These and similar units have an excellent record for *Anopheles* control on impounded water in the United States and in similar breeding areas throughout the world. The Authority's interest in aircraft sprays began simultaneously with the development of the exhaust generator, but initial emphasis was placed on the latter for larvicidal operations. The present revival of the study of nozzle atomization is due to:

1. Current expansion in the use of airplane larviciding to include smaller and less accessible areas since such areas frequently make it desirable in the interest of safety to increase the height of flight, which noticeably decreases the recovery of particles dispersed by aerosol generators.

2. The need to make applications during adverse weather conditions of wind and thermal instability in order to use available equipment efficiently since treatment with aerosols during unfavorable weather results in sparse and spotty distribution.

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3. The possibility of utilizing wing-tip nozzle positions to secure flatter and wider swaths.

4. Increased interest in the use of aircraft for applying 2,4-D and other herbicidal sprays.

5. The possibility of securing certain economy of installation and maintenance with the use of simple spray nozzles as compared to the more complicated exhaust generator.

6. The increased safety factor introduced by eliminating the constricted exhaust generator which has been claimed to affect the operating characteristics of the aircraft engine.

This report is concerned primarily with basic studies on the characteristics of the nozzle in breaking up liquids and the application of this information to the airplane distribution of larvicides for the control of *A. quadrimaculatus* larvae.

METHOD OF MEASURING PARTICLE SIZE OF SPRAYS

When liquids are atomized by means of a nozzle or an airstream, the particles produced are of various diameters. A knowledge of the frequency and size distribution of the droplets produced is of great importance in the study of liquid atomization when the results are to be applied to a specific problem. It has been customary to collect representative samples of the droplets produced by an atomizing device by means of either settling or impacted (waved) slides. The numbers of particles in various arbitrary size categories are counted, after measuring the spread of the liquid on clean or specially coated glass slides and determining the spread factor (Yeomans, (1949)) for the liquid under consideration. These data are then translated into some centering constant descriptive of the spray such as the average diameter or the median mass diameter (MMD). It has been found that the number of droplets counted should never be below 200 and extreme care must be taken to insure that a true sample of the spray spectrum is obtained.

During World War II, fundamental studies upon the atomization of liquids were undertaken by Division 10 of NDRC under the direction of Dr. H. F. Johnstone of the University of Illinois. At that time it was apparent that meager information was available on the particle sizes produced by atomizing devices. Lewis et al. (1945) uncovered the work of two Japanese engineers, Shiro Nukiyama and Yasusi Tanasawa, which has become quite useful in studying atomization problems. Their basic formulae may be applied to any atomization device, but were developed primarily for air atomizing nozzles. The empirical equation as analyzed by Dr. Johnstone's group accounts for the atomization in the simple nozzle through two steps. The first stage occurs within the body of the nozzle where the liquid emits from the orifice as a high velocity sheet. The second stage is the interaction of the high velocity sheet with the air which produces the ultimate breakup. By making certain experimental measurements for particular types and construction, such as hollow cone, flat, or cylindrical spray designs, fairly close predictions as to the particle sizes produced were made. The application of the formulae to spray nozzles in the range desired for the Authority's operation presented some interesting complications, mainly due to the direction of the nozzle with reference to the direction of aircraft movement.

The use of the Japanese work introduces a new centering constant for the spray, the D_0, which is measured in microns. The D_0 is the diameter in microns of a single droplet with the same ratio of surface to volume as a representative sample of droplets in the spray. It may be written simply as:

$$D_0 = \frac{\Sigma\, d^3 \Delta n}{\Sigma\, d^2 \Delta n},$$

where Δn denotes the number of drops, the diameters of which are d.

This equation for D_0 would be satisfactory if a sufficiently close interval of recording drop sizes were employed, and if the data could be collected without skips in the various size groupings, especially in the larger diameters. Fortunately, the second Japanese empirical equation expresses the relationship existing between the number and the diameter of particles in the spray spectrum. The differential equation for the distribution curves is presented as follows:

$$\delta n = a d^2 e^{-bd^q} \delta d,$$

where δn is the derivative of the number of droplets with respect to diameter d; and a, b, and q are constants. Further, the fitting of count data to this equation has been simplified by rewriting it in logarithmic form:

$$\log \frac{\delta n}{d^2\, \delta d} = \log a - \frac{bd^q}{2.3},$$

which can be recognized as an equation of a straight line having the slope equal to $b/2.3$. Therefore, the basic steps for determining the value of q, and finally D_0, for a given droplet count are simple and straightforward. A plot of the following will give a straight line when q is properly selected:

$$\log \left(\frac{\Delta n}{d^2\, \Delta d} \right) \quad \text{vs.} \quad d^q.$$

where d = drop diameter in microns
Δn = number of drops of d
Δd = drop diameter interval

The D_0 of the spray may be determined from the slope ($b/2.3$) of the plotted points most nearly approaching a straight line. The basic derivation of D_0 as a function of b is presented briefly below.

Since by definition,

$$D_0 = \frac{\int_0^\infty d^3\, \Delta n}{\int_0^\infty d^2\, \Delta n},$$

the value of Δn may be substituted from the drop-distribution formula and integrated to the following general form:

$$D_0 = \frac{\int_0^\infty d^5\, e^{-bd^q}\, \delta d}{\int_0^\infty d^4\, e^{-bd^q}\, \delta d} = \frac{\frac{(6)}{(q)}!}{\frac{(5)}{(q)}!} \times \frac{5}{6} b^{-1/q}.$$

The mathematics of the number-droplet relationship have been reduced to usable terms for computing D_0 after q, slope, and b have been found by the graphical method described above. The relationships expressed in the simplest form are as follows:

q	D_0 *as a Function of* b*
2	$1.5/b^{\frac{1}{2}}$
1	$5/b$
$\frac{1}{2}$	$110/b^2$
$\frac{1}{3}$	$4{,}080/b^3$
$\frac{1}{4}$	$213{,}000/b^4$
$\frac{1}{5}$	$14{,}300{,}000/b^5$

* $b = 2.3 \times$ slope.

Table 1 and Figure 1 give in detail the procedure for calculating q and D_0 from a droplet count of an aircraft thermal aerosol of 20 per cent DDT in Velsicol NR-70

TABLE 1

Calculation of q and D_0 for the thermal exhaust generator discharging 20 per cent DDT in Velsicol NR-70 at 0.1 lb. DDT per acre

DROP DIAMETER d, IN MICRONS	Δd	NUMBER OF DROPS, Δn	LOG $\frac{10^6 \Delta n}{d^2 \Delta d}$	d^2
5	5	23	5.26	25
10		26	4.72	100
15		48	4.63	225
20		26	4.11	400
25		38	4.08	625
30		22	3.69	900
35		18	3.47	1225
40		11	3.14	1600
45		6	2.77	2025
50		3	2.38	2500
55		1	1.83	3025
60		2	1.05	3600
65	5	1	.67	4225

discharged at the rate of 0.1 lb. DDT per acre. The exhaust generator is a very efficient atomizing device and is capable of producing drop spectra with q values of 2. It may be observed that when q is large, the droplets are relatively homogenous in size and the size-range relatively narrow, and when the droplet range is wide, the value of q is small.

The best straight line was obtained with $q = 2$; the slope $= \frac{4.79}{4770} = .001$; and $b = 2.3 \times .001 = .0023$. When $q = 2$, $D_0 = 1.50/\sqrt{b}$ (see D_0 as a function of b). Therefore, $D_0 = 1.50/\sqrt{.0023} = 31$ microns.

The advantages of using the plotting method for analyzing droplet size data are many. Considerable difficulty had been experienced in obtaining the close reproduction of samples of aircraft sprays using the more conventional MMD method of analysis. This was partially due to sampling error, but for the most part was due to the

sensitiveness of the MMD to the presence of a few larger sized droplets in the samples counted. Table 2 shows the comparison of the D_0 calculated by the plot method to the MMD for three samples of an aircraft spray.

It will be seen that relatively close agreement prevails between the calculated D_0

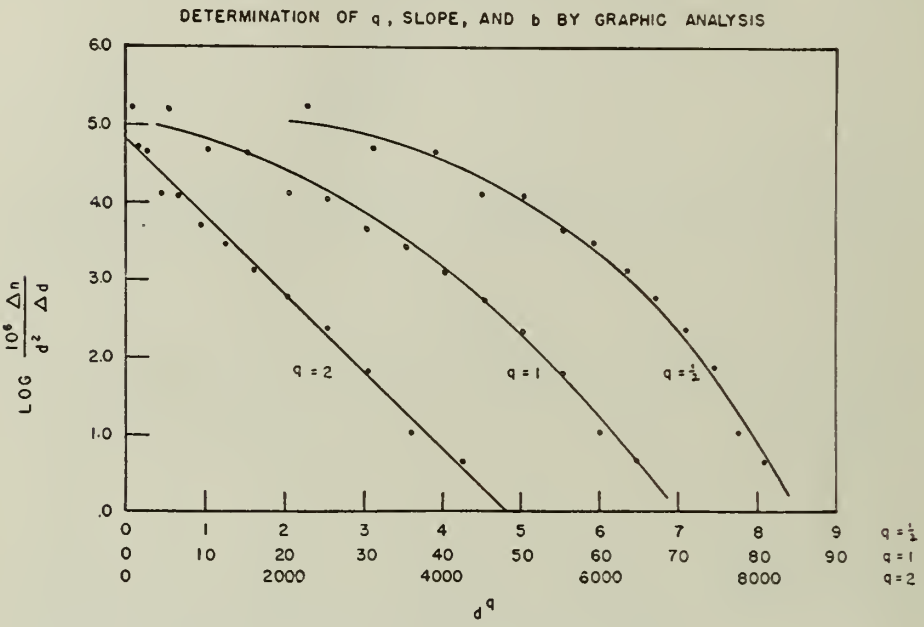

FIG. 1. Plot of Data as Required in the Calculation of D_0.

TABLE 2

Comparison of D_0 and MMD as methods of analysis for particle size data

2-8001 NOZZLES AT 110 PSI DIRECTED DOWNWARD

Sample Number	D_0 Microns	MMD Microns
1	124	260
2	127	150
3	107	140
*	125	160

* Summed as though all particles were contained in a single sample.

values while the recording of one, more or less large droplet has created considerable variation in the value of the MMD.

NUMBER AND SIZE OF PARTICLES IN RELATION TO CONTROL

A close relationship exists between the number and size of droplets, and the mass of insecticide released over, or recovered on, a breeding surface. A vivid demonstration of this fact is given in the following tabulation of the number of droplets capable of being produced per square inch of area from 200 cubic centimeters of 20 per cent

DDT solution (sp. gr. 1.08) distributed over one acre giving an application rate of 0.1 lb. of DDT per acre. The assumption is made that the total volume is completely broken up into a 5 micron diameter spray, a 25 micron diameter spray, etc.

Diameter in Microns	*Number per Square Inch*
5	494,000
25	3,610
50	490
75	146
100	62
200	8
300	2

The diameter of the particle affects the toxicity of the insecticide to larvae, the rate of fall, and the area of distribution. As long as individual droplets containing lethal concentrations of DDT for *Anopheles* larvae are adequately distributed over the breeding area, no increased kill should be expected by increasing the diameters of the droplets.

It has been previously calculated by Kruse and Metcalf (1946) that the minimum amount of DDT required to kill a fourth instar larva of *A. quadrimaculatus* is contained in about a 55 micron aerosol particle of which 25 per cent by weight is DDT. Therefore, a spray particle containing an equivalent dose of DDT must be about 69 microns in diameter. The difference in concentration between the aerosol (25 per cent) and the spray (20 per cent) particles is due to the evaporation of solvent from the 20 per cent concentrate as it passes through the exhaust venturi aerosol generator in the presence of the hot engine exhaust gases which consequently produces a more concentrated insecticidal solution. It follows that spray droplets of 70 microns diameter contain the minimum lethal dose and this size is considered to be the optimum for spraying devices. Field experiments seem to show that a mass recovery rate of 0.0005 lb. DDT per acre will provide at least 90 per cent larval control. Further, it appears that if the insecticide is well distributed, with several droplets occurring per square inch, good kill may be obtained with dosages as low as 0.0001 lb. DDT per acre. These recoveries and mortalities are for open breeding areas. Where heavy vegetative cover is encountered, a recovery of about 0.005 lb. DDT per acre on top of the vegetation will be required to provide sufficient numbers of particles to penetrate the barrier and give satisfactory kill beneath it. However, if the distribution does not average at least 1 droplet per square inch, control cannot be assured regardless of the mass recovered.

No atomizing device will produce a homogenous spray of particles having the same diameter. For purposes of analysis, a number of spray patterns of different particle size composition have been studied in hopes of setting some attainable limits for the ideal size. Extensive experience indicates that many particles under 50 microns diameter may drift away from the treatment area in spite of the downwash component of the aircraft. Under normal operation, only about 15 per cent of the total particles produced in aerosol actually reach the central 200 feet of the treatment area. Under optimum conditions of no drift, inversion, and at flight heights of 25 feet, about 20 per cent of the total number of droplets produced reach the treatment area. There-

fore, it is misleading to consider solely the total number of particles produced by a spray device. For this reason emphasis is given to the per cent of mass applied in the size group of 0–50 microns, 50–100 microns, and 100 microns and over. The importance of using the material most efficiently is apparent, because by minimizing overtreatment-peaks in the swath, significant savings in larvicides may be realized.

Table 3 has been prepared to show the relationship of particle size to the control of *Anopheles* larvae in both open breeding situations and under heavy vegetative cover. The column of expected recovery of droplets per square inch under normal conditions has been computed on the assumption that only 20 per cent of the droplets in the range 0–50 microns reach the treatment area. It must be recalled that the

TABLE 3

The analysis of particle size composition of various sprays to the control of Anopheles *larvae in open and heavy vegetative cover during optimum weather conditions*

D_0 MICRONS	q	PARTICLE SIZE COMPOSITION PRODUCED IN MICRONS*						CENTRAL 100 FEET OF SWATH				
		0–50		50–100		100–over		Average Recovery	No./Sq. In. Required† for Control		Per cent Recovery Required for Control	
		Per cent Mass	No./Sq.In	Per cent Mass	No./Sq. In.	Per cent Mass	No./Sq. In.	No./Sq. In.	Open	Heavy	Open	Heavy
30	2	82	296	18	2.6	0	0	61.6	10	100	16.5	100.0
50	1	50	181	45	6.5	5	0.1	42.6	2.5	25	5.9	59.0
70	1	18	65	58	8.4	24	0.4	21.8	1	9	4.6	41.0
80	1	15	55	60	8.7	25	0.5	20.2	1	6	5.0	30.0
90	1	11	40	53	7.7	36	0.6	16.3	1	4	6.1	24.5
100	1	11	40	44	6.4	45	0.8	15.2	1	3	6.5	20.0
120	½	10	36	36	5.0	54	1.0	13.2	1	1.5	7.6	11.4
150	½	9	33	26	3.8	65	1.2	11.6	1	1	8.6	8.6
200	½	3	11	25	3.6	72	1.3	7.1	1	1	14.0	14.0

* Based on a volume of 20 per cent DDT Solution (sp. gr. 1.08) equal to 0.1 lb. DDT per Acre.
† Open areas 1 droplet per sq. in. or .0005 lb. DDT per acre.
Heavy cover 1 droplet per sq. in. or .005 lb. DDT per acre.

numbers of particles recovered over a swath follow a normal distribution curve with many more droplets in the center than on the ends, and the larger the droplets the greater is the tendency to increase the total mass in the central portion of the swath.

In studying Table 3 it appears that any D_0 over 150 microns would represent much more mass than is required even in heavy cover, and the safety factor of adequate distribution becomes increasingly poor. On the basis of expected recovery alone, one could say that spray patterns with D_0 values from 70 microns to 150 microns should be entirely satisfactory. This is quite encouraging since many of the spray nozzles investigated fell well within this range.

The difficulty with the average-droplet-number-produced as a criterion, even after correcting for the loss through drift of the smaller fraction, is the lack of performance data on the distribution of droplets along the swath. All spray studies, including those on aerosols, show that many times more insecticide is recovered below

the point of release than is required to insure adequate control. The number of droplets needed compared to the number recovered also gives the impression that much more reliable control may be obtained with the larger diameters. This impression is questioned in the presence of heavy vegetation and under normal field application where the factor of penetration and dispersion is of great importance. The total swath distances in feet over which droplets of various size ranges are repeatedly able to reach during optimum weather conditions and at a flight altitude of 25 feet are as follows.

Diameter in Microns	*Swath Width in Feet**
0–50	260
50–100	200
100–over	120

* From wing tip nozzles; wing span is 40 feet.

From this swath information, Table 4 has been prepared which shows the problem of selecting sprays which will give adequate deposits and also the widest swath widths.

TABLE 4

Analysis of particle size composition of various sprays to the expected lateral dispersion under optimum weather conditions in open situations at flight altitudes of 25 feet

D_0	q	AVERAGE NUMBER PER SQ. IN. EXPECTED AT LATERAL DISTANCES IN FEET FROM WING TIP POINT OF DISCHARGE					
		40 Feet		80 Feet		120 feet	
		No.	Per cent Required for Control	No.	Per cent Required for Control	No.	Per cent Required for Control
30	2	61.6	16.5	61.6	16.5	59	17.0
50	1	42.6	5.9	42.5	5.9	36	28.0
70	1	21.8	4.6	21.4	11.5	13	75.0
80	1	20.2	5.0	19.7	12.7	11	91.0
90	1	16.3	6.1	15.7	15.9	8	—
100	1	15.2	6.5	14.9	16.8	8	—
120	½	13.2	7.6	12.2	20.5	2	—
150	½	11.6	8.6	10.4	24.0	1.8	—
200	½	7.1	14.0	5.8	43.0	0.6	—

Table 4 clearly shows what has already been demonstrated with the thermal aerosol. The smaller the diameter, the greater the swath width under optimum weather conditions. It also shows that efforts to widen the swath by increasing the number of the larger diameter particles above 90 microns will be futile.

In summarizing, the problem of size range of spray particles selected without regard to ability to penetrate heavy cover, is as follows:

1. The smaller diameter spray (0–50 microns) represents a tremendous number of droplets produced, which provides wider swaths but, also, requires more recovery in numbers of droplets per sq. in. and is quite sensitive to adverse weather conditions.

2. The 50–100 micron range appears to be the optimum for maximum recovery and swath width, and is not so sensitive to adverse weather conditions.

3. The 100 micron and larger particle size range gives the best mass recovery and is the least affected by adverse weather. It also represents the least number of droplets produced, gives less safety factor on distribution, and provides the narrowest swath width.

The larviciding equipment will be a factor in finally determining the spray range to be employed. For example, the helicopter is known to possess a downdraft of high magnitude which should favor the dispersion and recovery of the smaller diameter sprays. The fixed wing aircraft has much less downwash, and the spray particle range selected should be of an intermediate range of 50 to 100 micron diameters. If the larger size range is desired, the number of particles produced should be increased by increasing the volume discharged and by lowering the concentration of DDT in the solution.

Cutkomp (Unpublished manuscript), in comparing the relative merits of spray and aerosol deposition at application rates of approximately 0.1 lb. DDT per acre, found that effective deposits of sprays could be obtained when the aircraft was flown at a height of 50 feet, but that the practical limit for aerosol treatment was 25–30 feet, the ratios of total swath deposits being 12 to 1 at 30 feet and 24 to 1 at 50 feet. He reports also that greater recoveries of particles were obtained from the aerosols when more dense liquids were dispersed, while no increased recovery resulted from spray applications. However, much greater "peaking" of the deposit occurred at the center of the swath from the spray applications. This "peaking" was due to the location of the nozzles (8002 Teejet, Chicago Spraying Systems Company)—one on each wing and stabilizer tip and one in the center of the tail assembly.

With these preliminary concepts in mind, studies were conducted on spray devices on helicopter and fixed wing aircraft. It was reasoned that the most favorable diameter range would be produced by a spray having a D_0 of 70 or 80 microns and a q of 1. It will be noted that these sprays had the most number of droplets in the 50–100 micron range.

EFFECT OF PRESSURE AND PHYSICAL PROPERTIES OF THE LIQUID ON PARTICLE SIZE OF SPRAY

Studies were made to measure the effect of pressure on the particle size produced in a series of nozzles of both flat and cone spray construction. The technique employed for sampling from stationary nozzles required a spray chamber into which the spray was released in the absence of air currents and which would give no splatter of liquid from its surfaces. Earlier a small tower was used, but difficulty with splatter from the wide angle sprays, especially the hollow cone type, resulted in poor samples. For collecting the samples an elongated box with a slotted sliding top was used. The sampler held up to five 1-inch x 3-inch glass slides spaced at 6-inch intervals. As the spray was introduced into the chamber, the slotted panel was moved across the slide holders, the slides thus being exposed for a short period to the spray in a manner similar to that of a focal plane shutter on a camera. After a few trials, the speed of the panel could be adjusted to obtain the proper number of droplets.

The discharge pressure was held constant on the liquid by utilizing a compressed air source in conjunction with a pressure regulator. Various solutions were tried, but most of the data were obtained using a 20 per cent DDT solution in Velsicol NR-70.

The discharge volume of each nozzle was carefully calibrated at the several pressures employed. The nozzle orifice was measured and the dimensions plotted on cross section paper and the area of the orifice computed. It was found that the catalog (Spraying Systems Co., No. 22,(1943)) listings of the orifice diameters were based on approximations of the circular areas equivalent to the areas of the nozzle openings. It was not possible to determine the effective area of opening for the hollow cone type of nozzle due to the construction of the core and orifice units. Study was concentrated on the Spraying Systems Co. 8002 "Teejet" nozzle using three liquids possessing wide differences in physical characteristics. These liquids were 20 per cent DDT in Velsicol NR-70, 20 per cent DDT in Sovacide 544B, and water, at 25° C. In the case of water, carbon-coated slides were required for the particle size determinations.

Energy is required in any system which creates small droplets from large volumes of liquid. It is believed that the energy required is proportional to the amount of surface area created. In a stationary nozzle the only energy introduced into the system is that produced by the pressure head on the liquid. The amount of the static head actually converted into energy for atomization depends upon the discharge coefficient of the nozzle and the physical properties of the liquid discharged. Due to the small diameters of the orifices and the high velocities encountered, it is apparent that Reynolds number, R, is small over the range studied. Reynolds number shows that the role of viscosity in these experiments is of great importance.

It was found by plotting a function of the liquid velocity against the surface area produced under a given set of conditions, that a linear relationship existed. Figure 2 illustrates the effect of varying pressure and physical properties of liquid discharged upon the amount of surface area produced. The relationship of the D_0 diameter to its surface area is $1/0.166\ D_0$. From the curve of the 8002 Teejet nozzle, the following empirical equation for D_0 in microns was derived:

$$D_0 = 5300\ (\mu\sigma/V_e\rho)^{1.06},$$

where μ = Absolute viscosity in poises
σ = Surface tension in dynes per cm.
ρ = Specific gravity in grams/cc.
$V_e = Q/A$ = liquid velocity in ft./sec.

Computed D_0 diameters are tabulated below using the formula cited above for other Spraying Systems nozzles of similar construction. The material used was 20 per cent DDT in Velsicol NR-70.

DISCHARGE PRESSURE PSI	CATALOG NUMBER OF ATOMIZING NOZZLE					
	8001 D_0		80015 D_0		8002 D_0	
	Comp.	Obs.	Comp.	Obs.	Comp.	Obs.
20	300	460	330	367	262	270
40	181	168	186	266	150	145
60	145	151	140	168	117	110
80	135	124	124	121	108	103
100	115	121	—	—	100	93

It may be seen that fairly close agreement exists between the computed and observed diameters. There is much less reliability in the 20 psi data, for at this pressure the nozzles studied were on the threshold between atomization and streaming. In studying Fig. 2, it may be seen that viscosity is of greater importance than surface tension in the production of droplets of a given size. In the future, more attention should be given to the physical properties of DDT solvents in order that desirable limits of viscosity and surface tension be included along with specifications on specific gravity, boiling point, and solvency for DDT. It appears that the diameter produced is inversely proportional to the liquid velocity and viscosity. In several tests there was a noticeable leveling off in the degree of atomization in the 80 to 100 psi range, and generally, nozzles of the same capacity did not show any marked difference in particle size produced, whether or not they were of the flat or cone spray type.

Relationship of Nozzle Construction to the Particle Size of Spray

CATALOG NUMBER	TYPE	GPM AT 40 PSI, WATER RATING	D_0 AT 80 PSI*	D_0 AT 100 PSI*
8001	Flat	0.1	124	120
¼LN-6	Cone	0.1	132	127
8002	Flat	0.2	105	90
¼LN-12	Cone	0.2	128	93

* With 20 per cent DDT in Velsicol NR-70.

The fact that the smaller diameter orifices appear to produce somewhat larger droplet sizes is of interest. This may be explained on the basis that liquids of high viscosity encounter proportionally greater cross sectional resistance as the area of the orifice decreases which is concurrent with a dimunition of the discharge coefficient. In this connection a problem which warrants considerable study is that of the effects of physical properties of liquids upon droplet sizes produced in the course of their expulsion from nozzles.

EFFECT OF NOZZLE POSITION, DIRECTION, AND AIRCRAFT SPEED UPON PARTICLE SIZES OF SPRAYS

Knowledge of the performance of stationary spray nozzles cannot be applied directly to their performance on aircraft. Two main differences are: (1) additional energy of atomization is added by the rapidly moving air, and (2) the nozzle location and orientation has considerable effect upon the recovery of aircraft sprays, which in turn is the basis for the determination of the D_0 diameter.

Location of the Spray Outlet

Needless to say, aircraft used for insecticide application were primarily designed for other purposes. In general, however, most ships may be adapted for liquid dispersion if certain basic aerodynamic principles are understood. For spray dispersion, downdraft is of utmost importance in that it imparts an initial downward force to the particles, especially those of the smaller categories, which is many times the velocity of their natural subsidence rate. Downdraft forces originating from an air-

craft wing vary directly with the magnitude of the induced drag and inversely with the aspect ratio, i.e. (wing span)^2/wing area. The use of flaps will sometimes improve

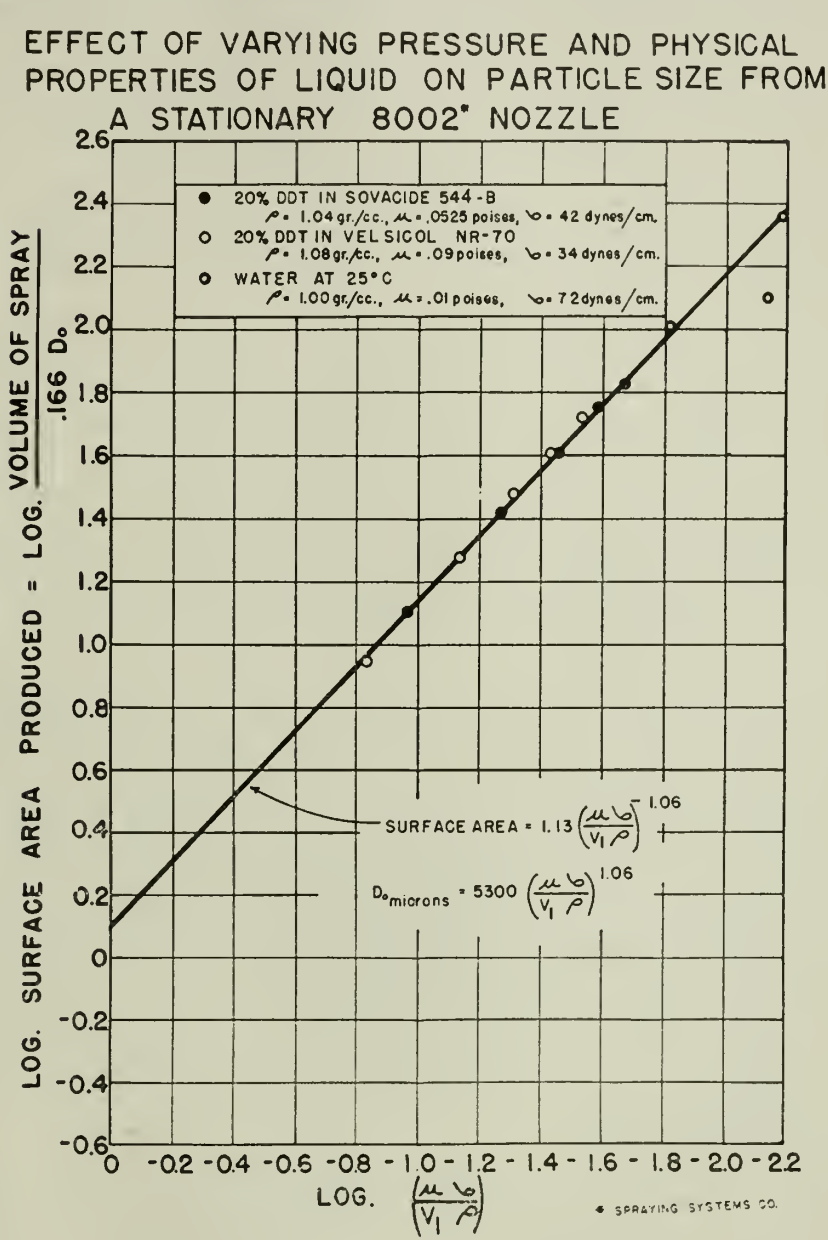

FIG. 2. The Relation of Liquid Physical Properties to Surface Area Produced.

the downdraft characteristics of aircraft so that greater penetration of spray droplets through vegetative cover will result. In this connection it must be kept in mind

that a diving aircraft has little or no induced drag while a climbing aircraft produces the maximum induced drag and downdraft. Johnstone et al. (1949) in analyzing the dispersion and deposition of aerosols, clearly show the relationship of drop diameter to deposition and efficiency of vegetative penetration. From their work it may be seen that a higher flight altitude is bound to dissipate much of the downward drive of the smaller droplets which was imparted by the airplane downdraft. When flying over vegetation, it is shown that the downdraft is rapidly dissipated and that following their penetration to a certain depth, the droplets are deposited only in accordance with their natural settling rates. This is confirmed in the field by the fact that good deposition and penetration is normally obtained in heavy but low vegetation such as "lizard tail" and the poorest beneath tall timber canopies having very little understory cover.

Spray outlets have been placed at many positions on the aircraft. Some locations have been along the trailing edge of the wing, on the wing tip, along tail booms, on booms forward and below the trailing edge of the wing, etc. It is evident that the location should be the point where maximum utilization of the available downwash will be obtained. Johnstone recommends a position forward and below the trailing edge at a point away from the turbulence of the wake. The nozzle position chosen for this study, as mentioned before, was at the extreme wing tip about 3/4 cord. The spray is introduced into the tip vortex which tends to throw particles first upward and out, then toward the path of the ship and downward. It was hoped that this arrangement would cause the heaviest droplets to be carried out farther and the smaller droplets to be carried downward.

A typical plot of the mass and particle recoveries at points 20 feet from the line of flight for a total distance of 240 feet, from an aircraft spraying experiment is given in Fig. 3. It is clearly apparent that a bimodal mass recovery curve was obtained. The nozzles were located on the wing tips, and with this nozzle arrangement, the same type of curve has always been obtained, though the peaks may be shifted to the right or left of the line of flight due to the effects of wind drift. This particular experiment was selected for discussion since the best meteorological conditions encountered during the season prevailed at the time it was carried out and the results can therefore be assumed to closely approximate those obtained under "ideal" conditions. That there was a slight though unmeasurable wind drift to the right of the line of flight is apparent upon comparing the height and breadth of the two peaks of the curve. The number curve likewise is bimodal but less distinctly so. A sufficient mass of DDT and number of particles to give a 100 per cent mortality (24 hrs.) of *A. quadrimaculatus* larvae were deposited from the left 80 feet across through the right 120 feet of the swath.

Figure 4 presents a graphic analysis of the relationship of the number and mass recovery at 20-foot intervals with respect to the total number of particles and total mass of DDT recovered across the spray swath. This analysis clearly shows that even though the large droplets (100 microns and over) make up less than five per cent of the total number, they account for over 50 per cent of the total mass of the spectrum recovered. In fact, since 100 per cent mortalities occurred at 80 feet to the left, at, and 120 feet to the right of the line of flight, the particles in the large diameter range actu-

ally represent an overtreatment and a consequent waste of larvicide. Figure 4 also shows that the particles in the intermediate range (50–95 microns) are distributed fairly uniformly across 200 feet of the swath, though their mass curve is bimodal with peaks about 40 feet to each side of the line of flight. The number and mass curves

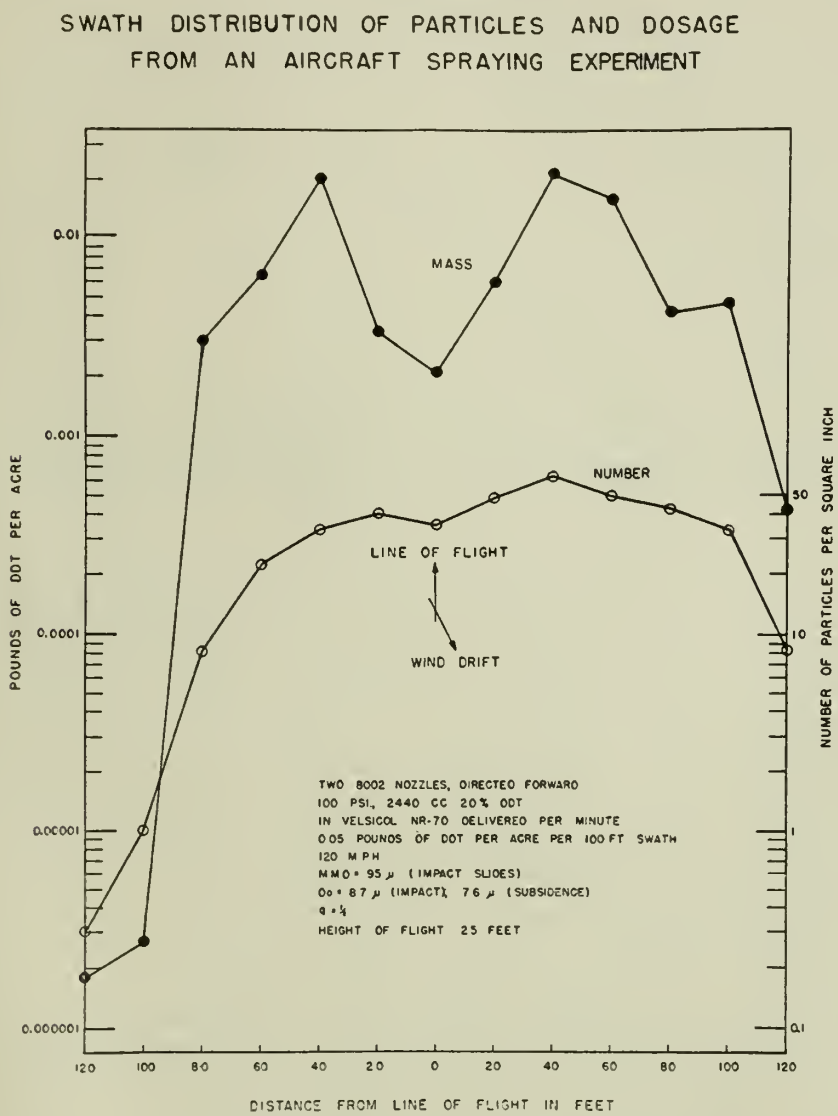

FIG. 3. A Typical Plot of Particle and Mass Recovery.

of the small droplet class (5–45 microns) show also that this class is subject to the effects of wind drift since the greatest number were recovered to the right of the line of flight.

Figure 5 presents an analysis of the number of particles and mass of DDT recovered with respect to the individual sampling stations located at 20-foot intervals across

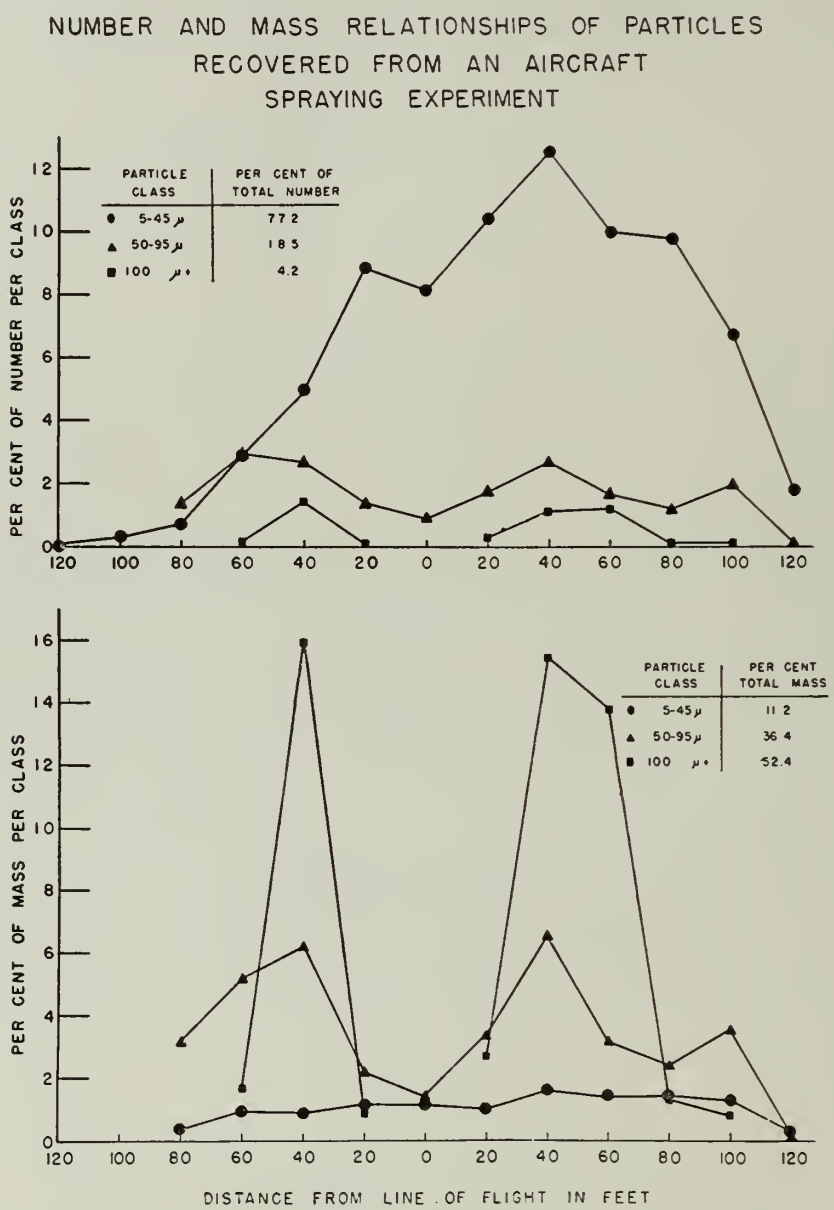

FIG. 4. The Relationships of Particles in Various Size Categories to the Total Amount of Insecticide Recovered.

the line of flight. This gives an insight into number and mass combinations which were responsible for the mortalities obtained at each sampling station.

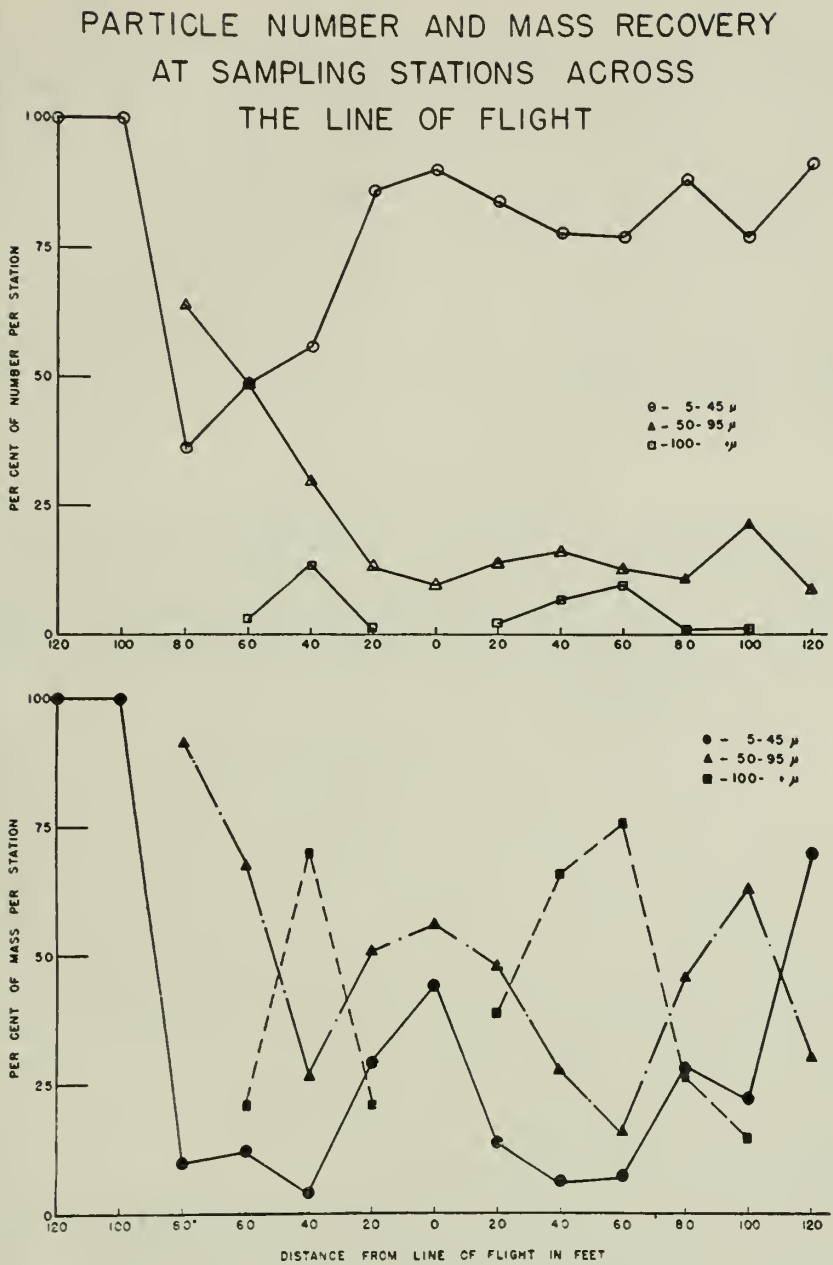

FIG. 5. The Relationships of Particles in Various Size Categories to the Amounts of Insecticide Recovered at Points Across the Spray Swath.

The foregoing discussion pertains to results obtained on an open range entirely devoid of vegetative cover which would hinder the settling of the spray particles. Efforts were made to study the penetration of sprays through heavy cover which

consisted of a scrub forest that was essentially open for about 20 feet above the ground capped by a dense leafy canopy about 10 feet thick. A very poor bioassay was obtained from the same application rate (0.05 lb. per acre) and nozzle combination as was utilized on the open range with the nozzles in either the heading or trailing positions. No better results were obtained when the nozzles were shifted to points about 10 inches below and 24 inches in from the wing tips in either of the two spraying directions. When the rate of application was increased to 0.1 pound of DDT per acre by placing two 8002 nozzles on each wing tip better control—100 per cent over 60 to 80 feet of the central 100 feet of the swath—resulted. Again, however, no clearcut distinction could be observed in performance between the heading and trailing directions. As yet, we have not arrived at a satisfactory solution to the problem of obtaining adequate spray particle deposition through high, dense cover, and it is planned that considerable effort will be expended the coming season to fill this deficiency.

Nozzle Direction and Air Speed

Nozzles are usually oriented in the trailing direction; however, experiments by the U. S. Department of Agriculture and the U. S. Public Health Service in fly control studies have reported a reduction in particle size by directing the nozzle downward. Following these leads a series of particle size measurements were made with the nozzle in the trailing, forward, and downward directions. The data for a series of Spraying Systems Co. nozzles at the same rate of discharge and at an aircraft speed of 120 miles per hour are tabulated below.

NOZZLE NO.	PSI	D_0 FOR NOZZLE DIRECTION IN FLIGHT			D_0 STATIONARY
		Forward	Downward	Trailing	
¼LN-6	100	93	163	266	127
8001	100	90	125	155	120
80015	55	118	152	224	165
8002	30	164	188	290	185

In all instances, the smallest diameter and q values were produced by the nozzle directed forward into the wind, intermediate when directed downward, and the largest diameter when directed trailing. Generally these observations seem to follow the basic theories of Nukiyama and Tanasawa, who maintain that the break-up of liquids after they leave the orifice is due to the interaction of liquid and air and is proportional to the differences in velocity between the two.

Since current interest in the use of the helicopter will call for speeds down to 20 miles per hour, data were collected on the 8002 nozzles in the forward and trailing directions over a range of speeds from 20 to 180 mph. A Vultee BT-13 was utilized in the 100 to 180 mph range, and an automobile for the slower speeds. On Figure 6 are plotted the effects of nozzle direction and varying air speed on the particle size produced. The 8002 nozzle was discharging the 20 per cent DDT in Velsicol NR-70 at a pressure of 100 psi. If the theory of breakup by interaction between air and liquid is held, there should be a point on the curve for the nozzle in the trailing direction

where the liquid velocity will equal the air velocity. At this point, where the difference in velocities is zero, no atomization or very little would occur. It will be noticed on Fig. 6 that the data show an increase in diameter on the trailing direction up to about 80 miles per hour, after which the diameters decrease as the speed increases. For the 8002 nozzle discharging the Velsicol solution at 100 psi, the balancing point is about 80 mph. Unfortunately, this point could not be accurately located in the field because 80 mph. was just beyond the range of safe automotive experiments and just below the safe flying speed for the aircraft. The expected large diameters were not found during the attempts made, but considering the difficulty involved, the results do show a fair indication of the relationship propounded. In the forward direction, it may be seen that the diameter became smaller as the speed increased. In the trailing direction, however, the diameter increased as the speed increased to a point where the liquid velocity and air speed were approximately the same. After this point the diameter varies inversely with the speed but in theory at least, will always be larger than those obtained with the forward direction.

Two, somewhat complicated, empirical equations have been developed from the data plotted on Fig. 6. The formulae are quite similar to the equation for predicting the D_0 of stationary nozzles which has already been presented. These equations are quite useful for predicting the D_0 of the spray produced by the 8002 nozzle using different solvents at varying pressures and aircraft speeds and with the nozzle being directed either forward or trailing. These equations have not, as yet, been field checked other than for the flat spray series 8001, 80015, and 8002. The equations have been applied to the 8002 nozzle over a range of pressures other than 100 psi and were found to check favorably with field observations. Figure 7 presents a series of curves predicting the D_0 of the spray produced over a wide range of pressure and air speed for both the trailing and forward nozzle directions. This plot is useful for demonstrating the breadth of spray characteristics that may be produced from a nozzle of a given design. It would be possible to superimpose upon these curves, other curves for different DDT solvents. Therefore, it may be seen that the particle size of a nozzle-produced spray on aircraft may be altered in four ways: (1) by physical characteristics of the liquid, (2) by discharge pressure, (3) by nozzle orientation, and (4) by speed of the aircraft.

APPLICATION TO THE CONTROL OF ANOPHELES LARVAE

It happened that the 8002 nozzle placed in the forward direction on the Authority's Vultee BT-13 larviciding aircraft produced D_0 diameters approaching the optimum size desired (about 70–80 microns). By merely turning the nozzle 180 degrees a greatly differing spray could be produced. This facilitated carrying out bioassays by permitting test runs to be made during substantially the same weather conditions with the same material at the same pressure settings. Figure 8 shows the differences in spray spectra to be expected with the nozzle directed either forward or trailing, other factors being constant. The mass delineation is of greatest importance in predicting the degree of recovery of a given spray.

Deonier et al. (1949) report that both fine sprays of 50–65 microns MMD (pro-

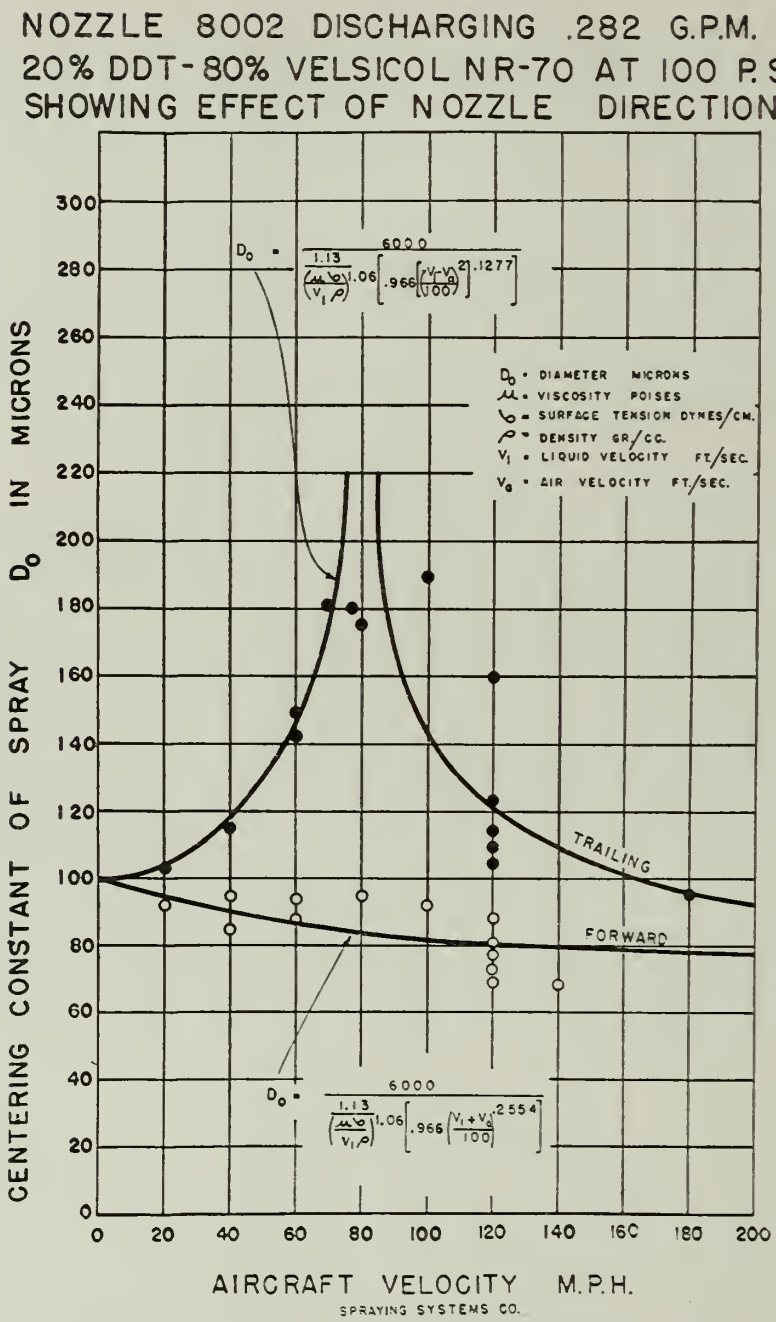

FIG. 6. The Effects of Airspeed Upon D_0 with Respect to Nozzle Orientation.

duced by an exhaust venturi generator) and coarse sprays of about 150 microns MMD (produced by a gravity-fed straight discharge pipe) will give excellent control of *Anopheles* adults and larvae. Since they observed a residual effect on larvae from the

coarse spray treatment (at rates of 0.3 and 0.6 lb. of DDT per acre) it must be assumed that excessive doses were applied. This heavy dose may have an injurious

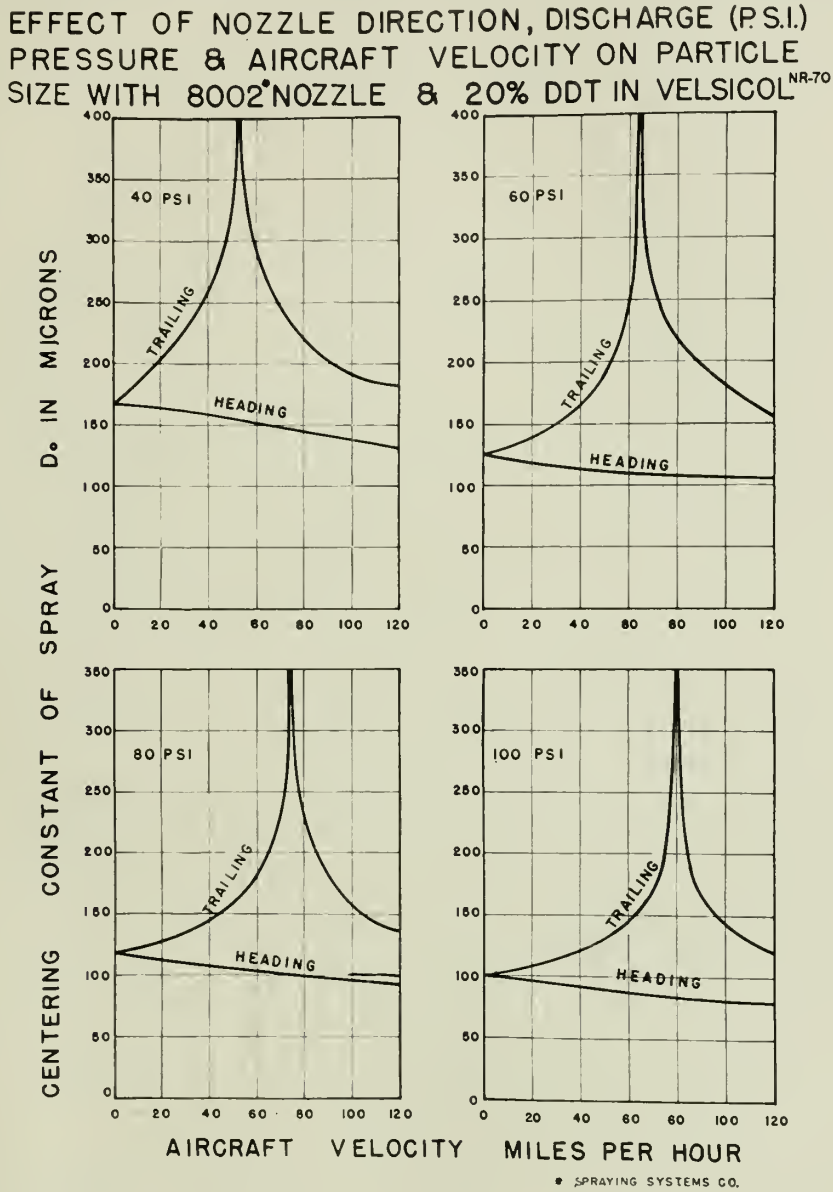

FIG. 7. The Effects of Discharge Pressure and Airspeed upon D_0 with Respect to Nozzle Orientation.

effect on wildlife, and on this account, care must be exercised to avoid overtreatment. It would be expected that the greatest mass deposit would occur from the 150 MMD spray.

In the present studies, the experimental data so far obtained indicate no constant difference between the orientation of the 8002 nozzle discharging 20% DDT in Velsicol NR-70 under 100 psi in open situations in either the heading or trailing direc-

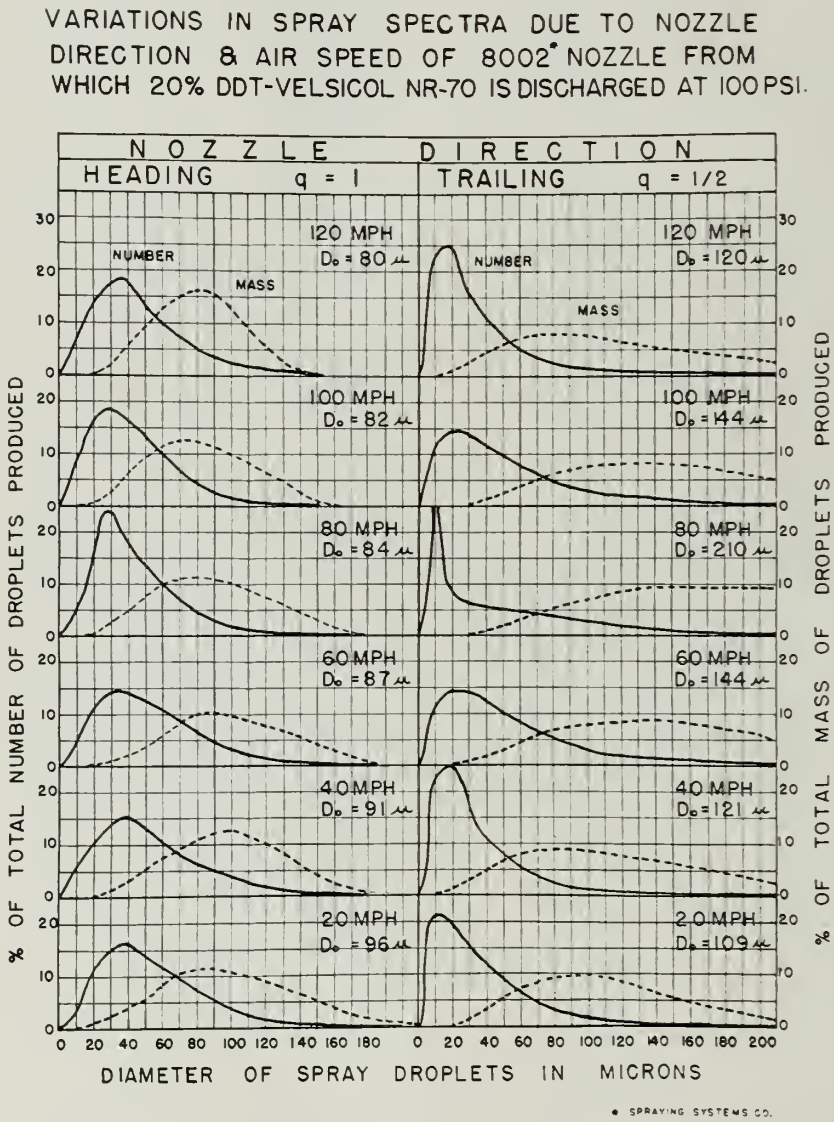

FIG. 8. Number and Mass Delineation of Spray Spectra Produced by the 8002 Nozzle with Respect to Airspeed and Nozzle Orientation.

tion other than in the total quantity and distribution of the mass of larvicide recovered across the spray swath. The total mass recovery from the trailing direction was about twice that from the forward direction. This was due to the greater num-

bers of particles of the large size range (100 microns and over) produced by the nozzles in the trailing direction. The mass distribution across the swath was less even, with considerably greater "peaking" occurring from this direction than from the forward direction. As previously pointed out in reference to Figures 3 and 4, "peaking" of the heavy droplets represents an overtreatment and a waste of larvicide, and may possibly lead to detrimental effects upon wildlife.

SUMMARY

The current interest in the application of aircraft distributed sprays has been primarily due to (1) the necessity of treating smaller and less accessible areas with a requisite increase in height of flight which predisposes a decrease in the number of aerosol particles deposited upon the breeding area, (2) the need to make applications during adverse weather conditions which likewise is a hindrance to the proper deposition of aerosol particles, and (3) an increased interest in the airplane application of herbicides.

It was found that a new centering constant for spray spectra, D_0, expressed the results more accurately than did the older mass median diameter concept, and a detailed description of the method of its application has been set forth.

Considerable emphasis has been placed upon the number, size, and distribution of particles produced by a spraying device and the relation they bear to the control of *Anopheles* larvae. The widest (about 260 feet) and most even distribution is obtained with particles 0–50 microns in diameter, but particles of this size range are subject to the effects of adverse meteorological conditions which make their deposition uncertain. Particles of an intermediate size range of 50–100 microns diameter have a distribution width of about 200 feet, and will be less evenly distributed than the smaller particles. It was determined that particles of a diameter of 70–80 microns would contain a dose of DDT lethal to *A. quadrimaculatus* larvae when a 20 per cent (w/w) concentrate of DDT in Velsicol NR-70 was dispensed. Large size particles, 100 microns and over, have a limited swath distribution of about 120 feet, and their numbers are relatively so few that it is questionable that adequate distribution (at least one particle containing a lethal dose per square inch) for control would be obtained. At least 0.0005 lb. of DDT per acre must be deposited to insure attaining at least 90 per cent control of larvae in open situations. Where heavy cover is present, about 0.005 lb. per acre will be required to insure the production of sufficient particles so that a toxic dose will penetrate the canopy.

A study of the effects of pressure and physical properties of liquids on the particle size composition of sprays showed that viscosity is of considerable importance. The D_0 of the spray spectrum appears to vary directly with functions of viscosity and surface tension and inversely with functions of liquid velocity (a function of pressure) and specific gravity. An empirical equation expressing these relationships is presented.

It is pointed out that the location of the spray outlet has a bearing upon the problem, since the location with respect to the downwash determines the amount of material deposited. Various locations have been suggested, but in this study, the nozzles were located on the wing tip at about the three-quarter cord. A plot of mass recovered from an experiment carried out during meteorological conditions approach-

ing the ideal showed a bimodal curve. The number curve more closely approximates that of a normal distribution curve. An analysis of the number and mass of particles by size-range categories showed a typical distribution according to class, with the particles in the 100 micron and larger diameter class actually being an overtreatment on the basis of bioassay. Efforts to obtain good larval kill beneath rather high, dense vegetative cover by doubling the volume of larvicide dispensed were not entirely successful.

Further evidence is given to support the theory of Nukiyama and Tanasawa that the breakup of liquids after they leave the orifice is due to the interaction of the liquid with the air and is proportional to the differences in velocity between the two. Data were obtained on the composition of spray spectra produced under pressures from 20 to 100 psi as influenced by speeds from zero to 180 mph with respect to heading or trailing nozzle-discharge direction. Successive decreases in D_0 occurred as the velocity increased when the nozzle-discharge was directed forward, but an increase in D_0 occurred as the velocity of the air approached the liquid discharge velocity when the nozzle-discharge was trailing. As the air velocity exceeded the liquid velocity, the D_0 decreased and approached that of the opposite orientation. Two empirical equations were developed to predict the D_0 of a liquid of known viscosity, surface tension and specific gravity, being discharged at a given pressure from an aircraft flying at a designated speed for the 8002 nozzle oriented either heading or trailing. The mass delineations of various spray spectra were discussed briefly.

ACKNOWLEDGMENT

The authors wish to take this opportunity to thank the pilots in the Transportation Section and the members of the Engineering and Biology Sections for their aid in carrying out this work.

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Calvin Chappel Kiker
1897-1949

Calvin Chappel Kiker, head of the Engineering Section, Malaria Control Branch, Tennesse Valley Authority, died October 4 of an acute heart attack at his home in Sheffield, Alabama.

Born August 24, 1897, in Blum, Texas, Mr. Kiker received his academic training in engineering at Texas Christian University. He started his professional career as highway engineer in Morris, Texas, later serving as city engineer at Mineral Wells, Texas.

In 1923 Mr. Kiker accepted a position with the engineering bureau of the Alabama State Board of Health where he remained for the next ten years. It was while associated with the late Engineer George H. Hazlehurst that Mr. Kiker became interested in malaria engineering and during his tenure of office made notable contributions to the engineering problems of malaria control.

With a reputation established in the field of malaria engineering, he came to the Tennessee Valley Authority on November 1, 1934, as associate sanitary engineer assigned to the Malaria Control Branch. The history of his activities in the malaria field during the next fifteen years and his contributions to the suppression of the disease in the Valley are well known to those interested in malaria. Those who have been privileged to work with Mr. Kiker have profited greatly through his sound judgment and common sense and his knowledge of malaria control gained through conscientious hard work.

At the time of his death he was a member of the American Public Health Association, the American Mosquito Control Association, and the National Malaria Society. For the past year Mr. Kiker served on the Editorial Committee of the latter organization.

Mr. Kiker was an exceptional person. From his early days with the Alabama State Board of Health he showed a fertile imagination and was indefatigable in the pursuit of his chosen profession. With it all was an inordinate modesty. His work was unusually productive of important results, as is evidenced by the development of permanent shore line improvement as a mosquito control measure in the Tennessee Valley. No single individual contributed more to the solution of the enormous malaria problem involved in the impoundment of the Tennessee River.

He made substantial contributions to the literature of malaria control. When his work came to an end, this Society lost a member of national reputation and his associates a beloved and honored friend.

STUDIES ON PORTABLE RESTING STATIONS FOR *ANOPHELES QUADRIMACULATUS* IN THE TENNESSEE VALLEY

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The Tennessee Valley Authority has used the adult densities of *Anopheles quadrimaculatus* in some seven hundred barns, sheds, and privies as a general guide to its malaria control program. However, during the past few years many of these structures have been rendered useless as mosquito collecting stations due to the application of DDT residual sprays for the control of adult mosquitoes. Therefore, it became necessary to seek a substitute type of resting station to serve as a general index of anophelism for areas bordering our reservoirs. The present paper summarizes our studies on different types of portable resting stations during the 1949 season.

RESTING STATIONS USED IN STUDY

Various types of portable resting stations and traps were considered, but for the present studies emphasis was placed upon modifications of the unbaited privy-type station and upon nail kegs. The unbaited privy-type station has been used successfully as an index of *A. quadrimaculatus* in certain areas in the Southeastern United States (Carpenter et al., 1946). The nail keg type has been studied in the Tennessee Valley and was found to be an effective resting station for *A. quadrimaculatus* (Smith, 1942). However, additional experience has indicated the need for a substitute as the nail keg has not always appeared to have sufficient sensitivity to measure low anophelism. Therefore, comparative studies were undertaken to measure the effectiveness of modified privy-type resting stations and of nail kegs where the two types were in direct competition along with a hollow tree for the existing adult anopheline population. The studies were all conducted under conditions of high anophelism at Blackwell Swamp, Wheeler Reservoir. High anophelism for the studies was desirable in order to secure a more sensitive measurement of the movements of the anopheline population as a whole.

The resting stations used in the studies were of three main types: (1) modified privy, (2) weathered nail kegs, and (3) an old hollow tree which in previous years often harbored as many as 3,000 adult *A. quadrimaculatus* per day. The dimensions of the privy-type house were 4 feet x 4 feet x $6\frac{1}{2}$ feet. Each wall was divided horizontally into quarters, each quarter being covered by a removable panel so that studies could be made of the entry of the mosquitoes at various levels (Fig. 1). Ingress baffles or trapping sections, similar to those employed by Bates (1944), were made of screen wire with a narrow horizontal slit which permitted the mosquitoes to enter the inside of the trap (Fig. 2). These baffles were so constructed that they could be inter-

FIG. 1. Basic type of demountable catching station showing four removable panels on each face with lower west panel removed.

FIG. 2. Demountable catching station equipped with ingress baffle on west side and with lower west panel removed.

changed with the removable wall panels. A general design of the privy-type resting station is given in Fig. 3.

In designing the privy-type resting station every effort was made to include features which would be attractive to adults of *A. quadrimaculatus* as based upon an intimate

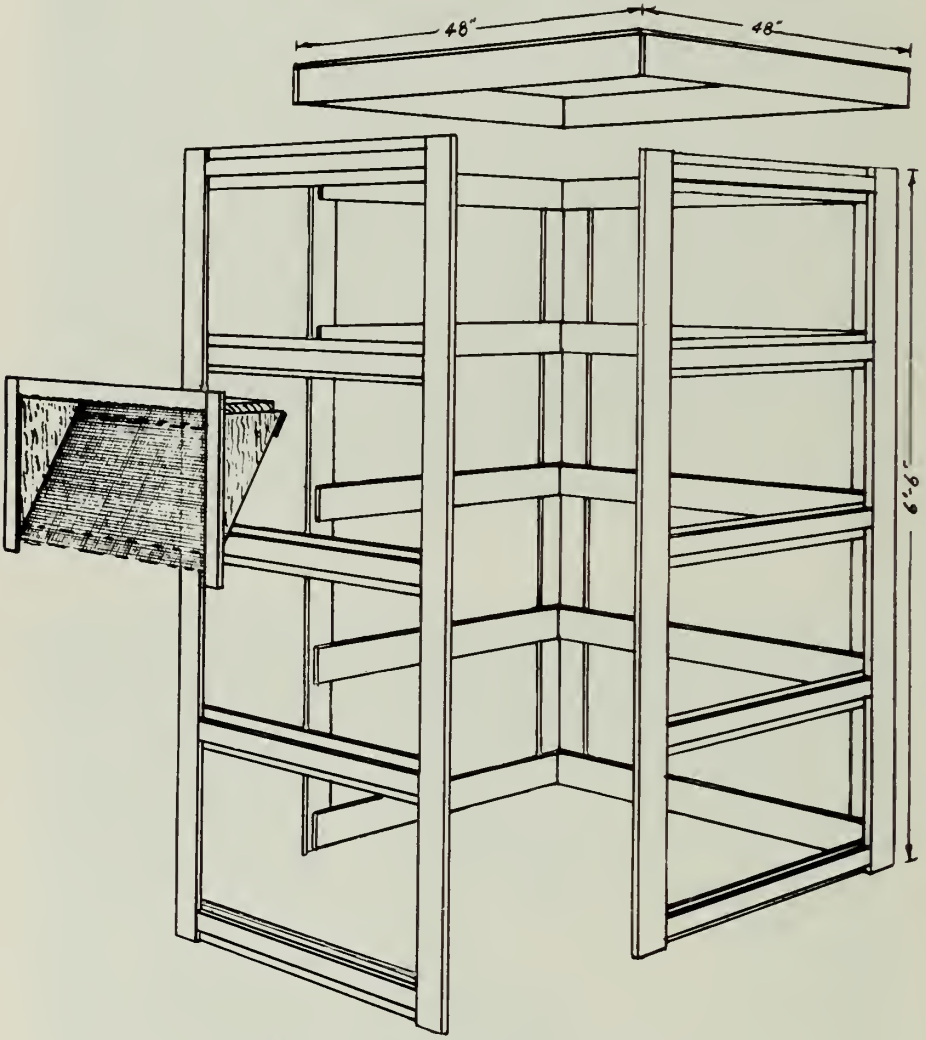

FIG. 3. A general design of the privy-type resting station.

knowledge of their habits. This appeared to be particularly important in view of the fact that the use of domesticated animals as bait was not feasible for our purposes. Desirable features included the use of a non-repellent construction material, a slightly roughened and darkened interior with a relatively large surface for resting, and protection from the adverse influences of wind and sun. Along with these features, it was deemed desirable that the trap be relatively inexpensive; be easy to transport, as-

semble, and disassemble; be easy to inspect and sample; and be durable enough to withstand exposure to the elements. On the basis of these considerations, a weatherproof cellulose board[1] of $\frac{5}{8}$-inch thickness was selected for construction of the traps. This weatherboard is coated on the outside with a granulated mineral and the inside is lined with a black moisture-proof backing of asphalt-like material. Laboratory tests showed that this type of board was non-repellent to adults of *A. quadrimaculatus*. The cellulose board also had the advantage of being pliable and hence could readily be bent into the desired position in assembling the traps.

METHOD OF STUDY

The studies were carried out in the tupelo forest at Blackwell Swamp in an area known locally as "The Bluehole." Six privy-type traps were placed at 15-foot intervals "parallel" to the shoreline within the tupelo forest. The hollow tree, of which mention has been made, was about 10 feet from the two center privy traps. This tree is one of the best natural resting places for *A. quadrimaculatus* that is known in the Tennessee Valley. Four nail kegs were placed on the ground between the demountable stations or privy traps. Thus, the nail kegs, privy traps, and hollow tree were all in close proximity to one another.

In these tests, at least three traps and three kegs were used for each series of conditions, and each study was replicated at least three times. That is, for each series of variations in trap set-up, the population was sampled on at least three separate days; the traps, the kegs, and tree all being sampled on the same day. All adult mosquitoes collected from inside each privy-house trap, from the baffles, from each keg, and from the hollow tree were kept separate. The collections were identified and tabulated with respect to species and sex. All collections were made before noon because these hours fall within the normal resting period for *A. quadrimaculatus*. Observations indicated that practically all of the adults were in the resting stations by the time of official sunrise even though all of the traps were empty one hour earlier. The mosquitoes usually left the traps from about 30 minutes to one hour before sundown even though they usually do not begin to feed until dusk.

COMPARATIVE EFFECTIVENESS OF RESTING STATIONS

Effectiveness of Privy Trap, Hollow Tree, and Nail Keg

The results obtained with various modifications of the privy-type trap, the nail keg, and the hollow tree are reported in Table 1. In each series of tests the privy-type of trap yielded the highest average catch of *A. quadrimaculatus* per station per day. The different types of resting stations in decreasing order of effectiveness were found to be the privy trap, the hollow tree, and the nail keg. The average catch per station per day from the privy-type trap ranged from $1\frac{1}{2}$ to 7 times greater than catches from the hollow tree and from 6 times to 24 times greater than catches from the nail kegs. On the other hand, if the modification in the privy-type trap made it less satisfactory, its adult population decreased with a resultant increase in the populations of the nail kegs and hollow tree. For example, when all panels of the traps were closed

[1] Celo-Siding, Celotex Corporation, Chicago, Illlnois.

(Table 1, Condition 3), ingress was afforded to mosquitoes only through the narrow slit at the top of the baffle, and they apparently preferred to rest in the other stations than to enter the privy traps.

Effectiveness of Various Privy-Type Trap Modifications

Field tests with various modifications of the privy-type trap were made using both nail kegs and the hollow tree as reference points for comparisons of effectiveness (Table 1). On this basis, the privy-type trap with the lower west panel removed and provided with a baffle on its west face was found to be the most efficient combination tested (Fig. 2). This combination captured more than twenty times as many *A. quadrimaculatus* as the nail keg on the average catch per station per day basis in

TABLE 1

Summary of catches of A. quadrimaculatus *in different types of resting stations*

CONDITION	NO. OF STATION DAY COLLECTIONS		AVG. CATCH PER DAY		RATIO OF POPULATION IN TRAPS TO POPULATION IN KEGS (APPROX.)
	Trap	Keg	Trap	Keg	
1a. Lower west panel off with baffle on west	24	18	394	20	20 times
1b. Two lower panels off on all sides, no baffles	24	18	214	20	11 times
2a. Lower west panel off with baffle on west	12	12	316	13	24 times
2b. Lower west panel off, no baffle	11	12	161	13	12 times
3. All panels on, entry only through narrow slit of west baffle	36	24	326*	87	4 times
4. Lower west panel off, baffle on west side	24	16	423	28	15 times
5a. Lower west panel off, baffles on east and west faces	12	16	620	42	15 times
5b. Lower west panel off, no baffles	12	16	298	42	7 times

* 96 per cent collected from the exterior surface of the baffle; 4 per cent from the interior of the trap.

In the combinations cited above, the a and b series sampled the same populations.

For each combination, the privy-type traps captured on the average of 1½ to 7 times the number of *A. quadrimaculatus* as the hollow tree.

paired series of tests. The combination with the lower west panel removed and with the baffle on the west face was found to be about twice as effective as the trap without the baffle but with the lower west panel removed (Fig. 1). The trap with two lower panels removed on each side and minus baffles was also found to be about half as effective as the most efficient combination. The presence of east and west baffles on the open resting station appeared to offer no special advantage over the single west baffle (Table 1).

Early in the studies it was noted that the adults showed a tendency to congregate to the west of the traps while seeking a resting place during the early morning hours. For this reason, it was felt that the entrance to the trap should be placed on the west side and this was used in the experiments both with respect to the single bottom opening and the location of the baffle. To test this, the privy-type traps with the lower west panel removed were set up with both east and west baffles to determine

the percentage population on each. The results from 12 trap-day collections from the baffles showed a total of 867 mosquitoes from the east baffles and 1,674 from the west. This suggests that the west surface is twice as attractive as the east due to the shading effect of the trap from the early morning sun. Thus, it appears that the entrance and baffles are most effective if placed on the west or on the morning shady side of the trap.

Traps which were provided with a west baffle and lacking the lower west panel showed about 71 per cent of the population to be in the interior of the traps while about 29 per cent rested on the baffles (Table 2). This fact raised the question as to whether the mosquitoes were moving into the trap through the horizontal slit or were entering mainly through the open lower west panel. To test this, traps having all panels in place were equipped with west baffles so that the horizontal slit of the

TABLE 2

Catch of A. quadrimaculatus *on baffles and inside box of baffled traps with and without lower west panel removed*

ARRANGEMENT OF TRAP	TOTAL A. QUAD.	PER CENT INSIDE	PER CENT ON BAFFLE
Baffle slit only entrance, lower west panel in place	11,749	4	96
Baffle slit on, lower west panel removed	23,389	71	29

TABLE 3

Catch of A. quadrimaculatus *by station type*

STATION TYPE	TOTAL NO. OF A. QUAD. IN STUDY STATIONS	PER CENT FEMALES
Privy House Trap	53,049	35
Nail Keg	3,723	30
Hollow Tree	3,481	37

baffle was the only portal of entry. Eighteen day collections were made from the baffle and from the interior of the trap (Table 2). Of the 11,749 anophelines captured only four per cent were collected inside of the trap while the remaining 96 per cent were found resting on the baffle. These results show that *A. quadrimaculatus* does not enter an unbaited trap readily through the baffle slit even though the baffle is an attractive resting place. They also suggest that in the tests summarized in Table 2 that most of the adults moved into the trap through the lower west entrance. Baiting the baffle slit trap with the collector frequently caused the inward movement of several female mosquitoes which were resting on the baffles. Presumably, bait would increase the efficiency of the trap.

TYPES OF MOSQUITOES IN RESTING STATIONS

Male and Female A. quadrimaculatus

The privy-type trap, the nail kegs, and the hollow tree all proved to be favorable resting places for both male and female *A. quadrimaculatus* (Table 3). The much larger

percentage of males observed in these studies is attributed to the close proximity of the stations to the breeding area. Many females leave the breeding area in search of blood meals, whereas the males remain closer to the breeding grounds and generally feed on nectar. While males commonly do not leave the breeding area, males were collected on "high ground" as far as one-quarter of a mile from the nearest breeding site during the course of these studies. About two-thirds of the population captured from the baitless types of traps placed near the breeding area may be anticipated to be males.

Other Anophelines

During the sampling period, 448 specimens of *A. crucians* were collected from the resting stations. Approximately 86 per cent of these were taken from the privy-type stations and the remainder were from nail kegs with the exception of a single specimen from the hollow tree. *A. crucians* showed an apparent preference for the demountable stations from which the lower two panels had been removed from each side. The collections also included 289 specimens of *A. punctipennis*, with approximately 93 per cent being taken from the demountable stations and the remainder from nail kegs. A single adult female of *A. walkeri* was collected in one of the nail kegs. None of the station types studied were attractive to *A. walkeri* which was found to be abundant during periods of the study by other sampling techniques.

Culicines

Fifteen species of culicines were taken in the collections and these species occurred mainly in the privy-type traps and the nail kegs. The following species were taken most frequently: *Culex apicalis*, *C. erraticus*, *Culiseta melanura*, and *Uranotaenia sapphirina*. The remaining eleven species were infrequent to rare and these were: *Aedes sticticus*, *A. thibaulti*, *A. triseriatus*, *Culex quinquefasciatus*, *C. restuans*, *C. salinarius*, *Mansonia perturbans*, *Psorophora confinnis*, *P. cyanescens*, *P. ferox*, and *P. varipes*.

SUMMARY

Studies of different types of portable resting stations were made during the 1949 season. These indicated that the privy-type trap equipped with a west baffle and with the lower west panel removed was the most efficient combination. The traps which were set up near the breeding area were sensitive in detecting both male and female adults of *A. quadrimaculatus*.

ACKNOWLEDGMENT

The author wishes to express his thanks and appreciation to the members of the Engineering Section and Biology Section for their cooperation and help in making these studies possible. Special mention should be made of the help of Mr. John D. Pitchford, Jr. for his tireless services in conjunction with the field studies.

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THE EXPERIMENTAL TRANSMISSION OF *PLASMODIUM FALCIPARUM* BY *ANOPHELES ALBIMANUS*

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Anopheles albimanus has been found in nature infected with *Plasmodium* and has been frequently infected experimentally with human malaria (Simmons, *et al.*, 1939). Circumstantially, it has been indicted as an important malaria vector in some of the

TABLE 1

Data relative to the transmission of Plasmodium falciparum *by means of* Anopheles albimanus *mosquitoes*

PATIENT NUMBER	MALARIA STRAIN	INFECTED MOSQUITOES BITING PATIENTS		RESULTING INFECTION IN PATIENTS
		Number	Average sporozoite intensity§	Prepatent Periods Days
G178	Santee-Cooper†	2	2.5+	13
G177	Santee-Cooper†	2	2.5+	No infection
G225	El Limon‡	16	3.3+	11
G226	El Limon‡	12	3.7+	9
G243	El Limon‡	6	3.2+	13
G231*	El Limon‡	4	3.5+	10
G235	El Limon‡	13	3.5+	12
G247	El Limon‡	5	3.4+	10 or 11
G250	El Limon‡	18	3.0+	12
G251	El Limon‡	18	3.5+	13
G253	El Limon‡	24	2.2+	13

* White patient; all others were negroes.

† From South Carolina.

‡ From Republic of Panama.

§ The sporozoites were recorded under the following intensity groupings: 1–9 sporozoites 1+; 10–99, 2+; 100–999, 3+; 1,000 and over, 4+. In the above table, these intensity groups were averaged, i.e., the average of 1+ and 2+ is expressed as 1.5+.

regions in which it occurs, but we can find no report in the literature of actual experimental proof of its ability to transmit malaria. To complete the incrimination of this mosquito, we are reporting here several instances in which *Plasmodum falciparum* was actually transmitted from person to person.

An American strain (Santee-Cooper) and a Panamanian strain (El Limon) of *P. falciparum* were used. The mosquitoes were from a strain of *A. albimanus* from Panama which had been colonized by Dr. L. E. Rozeboom (Rozeboom, 1936). We are indebted to the Army Medical School for the ova from which our colony was established in 1946.

The data on the transmission attempts are shown in table 1. Of the 11 attempts to transmit *P. falciparum*, 10 were successful. No significance is attributed to the one failure (Patient G177), as the mosquitoes were not heavily infected. Failures often follow the use of small inocula of sporozoites with other species of mosquitoes.

The clinical attacks of *P. falciparum* in the patients infected by means of *A. albimanus* did not differ from attacks induced by means of *A. quadrimaculatus*.

SUMMARY

A Panamanian strain of *Anopheles albimanus* transmitted two strains of *Plasmodium falciparum*. One strain of malaria was from the United States and one from Panama.

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BOOK REVIEWS

"*Blakiston's New Gould Medical Dictionary.*" 1st Ed. HAROLD WELLINGTON JONES, M.D., COL. U. S. A. (retired), NORMAN L. HOERR, M.D., PD.D., AND ARTHUR OSOL, PD.D. 1294 pages with 45 pages of illustrative plates. The Blakiston Co., Philadelphia 5, Pa. 1949. $8.50.

Blakiston's New Gould Medical Dictionary is a completely new reference work based upon *Gould's Medical Dictionary*. It has been edited by a board of three, assisted by seventy-seven distinguished contributors who are specialists in their particular fields. The preface indicates that more than three hundred standard modern texts in the fields of medicine, surgery, and biology as well as recognized journals, yearbooks, and standard indexes were critically and systematically examined, and the publishers claim this is the first new medical dictionary in thirty-eight years.

It appears that an outstanding job has been done in the preparation of this new dictionary, and the editors and publishers are to be congratulated upon the appearance of this volume.—E. Harold Hinman.

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